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As this number goes to press, the world of science and literature is saddened by the death on 29 May 1960 of

JOHN FARQUHAR FULTON

Neurophysiologist, Historian, Teacher, and Man of Letters

The University loses a man of outstanding worth in his devotion to his work, his unselfish generosity in giving of himself to others, and his gift for intellectual stimulation. He brought great honor to Yale.

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THE YALE JOURNAL
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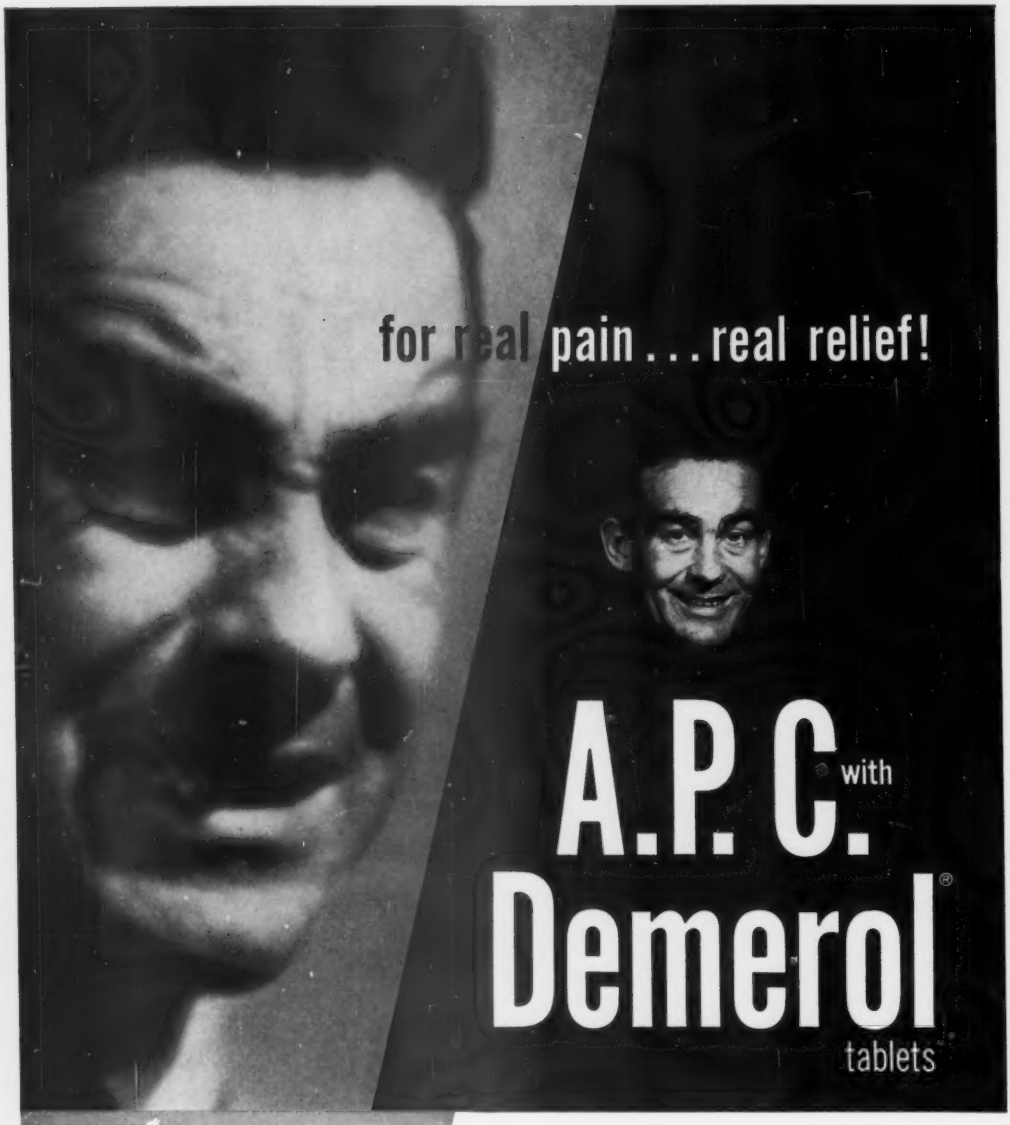

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Ross G. Harrison

J. S. NICHOLAS*

The Osborn Zoological Laboratory, Yale University

ROSS GRANVILLE HARRISON
1870-1959

It is most fitting that this number of *The Yale Journal of Biology and Medicine* should be dedicated to the memory of Ross Granville Harrison. His best known contribution to the field of medical research is the tissue culture method which is now being so importantly applied to the study of disease. This, however, represents but one aspect of his thought and interest in medical problems both at Yale and elsewhere.

Born in Germantown, Pennsylvania, January 13, 1870, he had his early schooling there. Later he attended the public schools in Baltimore and then Marston's University School before matriculating at The Johns Hopkins where he completed his undergraduate work, securing the A.B. degree in 1889. His graduate work at The Hopkins was with Professor W. K. Brooks, a teacher and scholar who left a lasting effect upon Harrison's life. Harrison's respect for Brooks was shown in the care with which he prepared his nerve outgrowth article for the Brooks Memorial Volume of the *Journal of Experimental Zoology*. He was granted his Ph.D. in 1894. During this graduate period Harrison had spent 1892-93 abroad working at Bonn with Professor Moritz Nussbaum. While studying there he met Ida Lange whom he married in 1896. He returned to Bonn and received his M.D. in 1899. As an instructor in the new Johns Hopkins Medical School he carried out his teaching along with the medical study on which his Bonn M.D. was partially based.

His associations with The Hopkins were always closely maintained. He started his teaching with Franklin P. Mall as the head of anatomy and he adopted Mall's method of teaching. He was intimately acquainted with the great, known as the Big Four of the new Medical School, Osler, Welsh, Halsted, and Kelly. In the Department of Anatomy he was associated with Warren Lewis, Florence Sabin, and Lewellys F. Barker in addition to Mall.[1]

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* Sterling Professor of Biology.

[1] Gertrude Stein was diverted in her quest for medical knowledge by this group; at that time she was working with Florence Sabin on wax reconstruction of the brain and brain stem of the newborn.

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Harrison after a year as lecturer at Bryn Mawr returned to The Hopkins to teach histology and embryology from 1896-1907 and was Associate Professor of Anatomy from 1899-1907 when he came to Yale as Bronson Professor of Comparative Anatomy. In his early years of teaching at Yale, Lewis Weed, later Dean of The Hopkins Medical School, and Stanhope Bayne-Jones, later Dean of the Yale School of Medicine, were his students as undergraduates.

Harrison's official relation with the Medical School during his first days at Yale is not immediately apparent. Probably the duties of building the Osborn occupied all of his spare time for he was conscientious about inspecting the construction of the building each day. His contacts in the Medical School, however, were numerous. George Blumer and Joseph Flint were Hopkins acquaintances and H. B. Ferris and Yandell Henderson both were friends. As given in the minutes of the Board of Permanent Officers, Harrison was sitting on the Board on October 20, 1914. At that time the preclinical work was given in the York Street building. A committee of the Medical Faculty was set up to consider moving anatomy to the Osborn, thus releasing sorely needed space for physiology and pharmacology.

By this time two of Harrison's Ph.D. students were instructing in the Department of Anatomy: Davenport Hooker, later Professor of Anatomy at the University of Pittsburgh, and H. Saxton Burr, now E. K. Hunt Professor of Anatomy Emeritus. S. R. Detwiler (former Professor of Anatomy at College of Physicians and Surgeons) and L. S. Stone, Bronson Professor of Comparative Anatomy, also have served the School. Harrison always felt that his students should know morphology and that taking human anatomy was the best way to acquire it. It served well for many anatomy departments throughout the country. The contributors to this memorial issue are a part of the Osborn Zoological Laboratory supply of anatomists trained under the Harrison regime.

Anatomy was not moved to Osborn; it was easier for the anatomists to go to the York Street Medical School where 'Pop' Ferris gave his wonderful lectures, broadly comparative in their scope and content. The medical students went to the Osborn for their embryology with Harrison and Reynald Spaeth.

Dr. Harrison had a large share of medical committee work and served at one time or another on 16 of these Committees. He was usually on every committee dealing with appointments, among these Milton C. Winternitz's to the chair in pathology and later to the Deanship. He remained a member of the Board of the Childs Fund after his retirement and was also a Trustee of the Rockefeller Institute.

His relationship to medicine was maintained on a national scale. He was in constant touch with Abraham Flexner during the crusade to better medical training and education and to a lesser degree with Simon Flexner, then director of the Rockefeller Institute, with whom he had become acquainted at The Hopkins. Both Flexners, and many others during his lifetime, greatly appreciated his ability to think things through to a logical end.

After retiring from Yale, he was Chairman of the National Research Council from 1938 to 1946. During this period the function of that body changed from peripheral adviser to government agencies on issues in which information was requested to an operating agency which sought to make the agencies aware of what scientists could do for government agencies' problems. Since science was doing most unusual things in connection with World War II, the entire focus of the importance of scientists as a group was intensified to a large extent through the efforts of Dr. Harrison and Dr. Jewett, then president of the National Academy of Sciences. This pair of minds cooperated in some of our major national policy decisions.

Harrison was appointed to the Science Committee of the National Resources Planning Board, one of the most progressive of the Government agencies. Headed by Mr. Delano with Dr. Elliot as Executive Secretary, it was a real working body with first-rate minds operating at high levels. The part of their planning which was to have tremendous significance had to do with the budgeting of manpower for the military. This led to an allocation program which the military were loath to accept, for it recognized that scientists could serve their country in modern warfare crises better when not in uniform and not subject to petty military restriction. The idea of the necessity for civilian national universal service instead of universal military service was at least partially accepted in Washington during this period. Without its acceptance even though reluctant, the Manhattan Project could not have been completed. In a large measure this was due to the forces, political and otherwise, that were advanced by the National Resources Planning Board. It remained a Policy Board throughout its existence and was politically smothered only after it had rendered a remarkable national service. Its Science Committee functions were taken over by the Office of Research and Development, the Manhattan Project, and the National Research Council. All these became operating agencies and, while there was not always the bilateral give and take between these groups that there should have been, Dr. Harrison and Dr. Jewett managed to coordinate both the information and the advances of the first two and to supplement them wherever possible.

At this time the lifeline of science was nearly broken by the college presidents' acceptance of an unwise call for universal sacrifice at the college

level. The Science Committee and the National Research Council working through Selective Service and the War Manpower Commission did much to avert the damage which would have resulted from the interruption of the training of scientists. In this, the cooperation of General Hershey, the head of Selective Service, was outstanding for he not only understood the problem, but with typical Hoosier craftiness invented the mechanisms by which scientists could continue their training, thereby maintaining a manpower reservoir which was so valuable during the last World War.

Dr. Lewis Weed was then Chairman of the Medical Division of the National Research Council. General Stanhope Bayne-Jones was in control of the Surgeon General's Office of Research. Through these men Harrison was in constant touch with the national picture of medical care not only for the armed forces but also for civilian needs. The production of penicillin in large quantities in this country was arranged through channels which had their origin with Harrison. Robert Coghill accomplished this with scientists from Dr. Howard Florey's laboratory working at the Peoria Laboratories where was produced a supply increasing in quantity during the entire war period. It was likewise due to Harrison's influence that Dr. Keefer was appointed to ration this antibiotic to take care of critical areas, both military and civilian. The supply, while limited, was utilized as completely as possible. Harrison also assisted the office of Medical Procurement by supporting their contention that medical personnel, which was in short supply, be utilized in the regions of greatest need where they could serve to greatest advantage.

Wherever Harrison touched medicine, whether through advice, teaching, training anatomists, or administration of war problems directly related to medicine, his contact made for professional betterment. It is, however, through his research that he made his greatest contribution to medicine, for the method which he evolved for the solution of the problem of nerve outgrowth was and is proving of the greatest value. The demonstration of the nerve origin changed the whole line of thought in neurology. The physiological integrity of the neurone rests upon the embryological facts which he demonstrated. The method itself is being used successfully in the present period in many fields. A Nobel prize was awarded for a modified application of his method, the growing of monkey kidney cells in quantity in tissue culture, which made the Salk vaccine possible on a commercial scale.

His later work is just as remarkable although it is not yet being applied to medical problems. This is the result of the same sort of critical planning; it led to the discovery of the rules which govern asymmetry. To those of us who have had the privilege of studying with him he passed the knowledge of the way the organism must be approached if questions are to receive definite

answers. It has sharpened the focus of our thought and has made us think of structure and function at molecular levels. His paper with Astbury shows that this approach to the asymmetry problem was very much in his thinking and, while the condition of preparation of his material did not present critical X-ray diffraction patterns, it was a step toward the current manner of investigation and showed that this is a possible method of attacking changes in molecular structure.

Dr. Harrison was a modest man, and the many honors which came to him in recognition of his work were received with such reticence that they were unpublicized. When he received his first Sc.D. from the University of Cincinnati in 1920 he said on opening the notification that this was his first realization of the aging process. This award was to be followed by many more: Dr. Phil. Nat., Freiburg; Sc.D., Michigan; Sc.D., Dublin; M.D., Budapest; Sc.D., Harvard; Sc.D., Yale; Sc.D., Columbia; LL.D., Hopkins; Sc.D., Chicago; and Dr. Rer. Nat., Tübingen.

He served as the only American president of the Anatomische-Gesellschaft and presided at the Königsburg meeting in 1937. In this country he was president of the Anatomists (1912-14), Naturalists (1913), Zoologists (1924), and Growth Society (1946-47). He was Chairman of the National Research Council (1938-46). His touch with science abroad was constantly maintained through his society memberships. These included the Royal Netherlands Academy of Sciences, the Norwegian Academy of Sciences, Accademia Nazionale dei Lincei, Royal Physiographic Society of Sweden, The Royal Society London, Royal Societies of Sciences, Uppsala and Stockholm, and the Zoological Society of London. He was a foreign associate of the Académie de Médecine Paris and correspondent of the Académie des Sciences, Institut de France. Among his honorary memberships is that of the Connecticut State Medical Society.

Three medals were presented to him: the Archduke Rainer Medal of the Imperial Royal Botanishe-Zoologische Gesellschaft, Vienna; the John Scott Medal and Premium of the City of Philadelphia; and the John J. Carty Medal of the National Academy of Sciences, U.S.A. Two other major honors must be recorded. He was the first American zoologist to be voted the Nobel Prize. It was never awarded since The Caroline Institute ruled that no awards be made in Physiology and Medicine during World War I (1914-18) and since the vote was in 1917, the award was never made.[3] In 1956, however, Dr. Harrison was awarded the Antonio Feltrinelli International Prize by the oldest of the academies, the Accademie Nazionale dei

[3] See *Nobel, the man and his prizes,* page 245, for the account of this action. Univ. of Oklahoma Press, 1951.

Lincei. This came after he was incapacitated by injuries sustained in an accidental fall from which he unfortunately never fully recovered.

He died on the last day of September 1959. It is seldom that one man can attain such true greatness. Every major effort was constructive and conspicuously successful. His contribution to biological thought is equivalent to that of Einstein or Planck in other branches of science.

RAYMOND F. BLOUNT*
ISABEL H. BLOUNT**

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THE RELATION OF THE PITUITARY TO GLOMERULAR STRUCTURE AND A HYPERTENSIVE STATE IN AMPHIBIAN LARVAE

The question of endocrine involvement in renovascular disturbances is still an open question, and any evidence that can be brought to bear upon the problem should be of value. In our work, in which additional pituitary transplantation has been performed in the amphibian embryo, certain striking similarities to a hypertensive state have been observed. In these animals there is a high level of pituitary hormone which is constantly, and physiologically, maintained. In these "triple pituitary" animals there is, as previously reported,[4] a vasoconstriction and an enlargement of the heart, accompanied by a slowed contraction rate. Although there have been no direct measurements of blood pressure, it is increased, as shown by the pulsatile character of the blood flow. That this is a good criterion is substantiated, particularly for the glomerulus, by the work of Hayman.[15] The kidney shows a series of changes thought by some to be characteristic of hypertension. The basement membrane of the glomerular tuft, as seen with the light microscope, is thickened[5] in these animals as was found by McGregor[20] and others in human hypertensives. Also in our work the reverse effects with a thinned basement membrane are found in the hypophysectomized animals and this is further corroborative evidence of the effects of the pituitary. In an attempt to reproduce these changes in the rat[5] we injected pitressin every hour during the day and every three hours at night for a period up to 65 days without effect. Since then Byrom[11] has obtained pathological changes in the kidney although he did not comment on the basement membrane. With other pituitary hormones and doses in keeping with the great requirements of the rat, changes might be brought about. The present paper will report further studies on the glomerulus of the amphibian.

MATERIALS AND METHODS

The observations here reported are upon sets of Amblystoma punctatum (Ambystoma maculatum) larvae. Each set consists of a "triple pituitary" animal with

* Professor of Anatomy.
** Research Associate.

two extrapituitary glands, a control, and a hypophysectomized individual. The operations were performed during the embryonic stage (Harrison's stages 28-31) as previously reported,[6] and the animals were raised as comparable groups, each having the same care. The various sets are of different ages but are generally in midlarval life. The animals were killed in Bouin's fluid and the entire body sectioned serially at 10 micra for general observations; for careful study and for the construction of models they were cut at 5 micra. Most of them were stained with Mallory's triple stain but some in azo carmine.

Several different observations were undertaken both for the mesonephros and for the pronephros. Glomerular volumes were determined by the paper cut-out method, using every section at a magnification of 500 as was previously reported.[7] The models were constructed at a magnification of 2,000. Drawings were made from the sections, such as shown in Figure 1, by projection of each one-micron of tissue thickness. This was done by using five optical levels within each of the five-micra sections. These were drawn first at a magnification of 1,000 on a transparent medium and then enlarged to 2,000 on a photographic enlarger. The models were constructed of wax in the usual manner.

The afferent arterioles of glomeruli were measured with an ocular micrometer under oil immersion at the point where the vessel passes through the capsule of the renal corpuscle. A large number of measurements was made, but for statistical evaluation one set of representative animals was studied thoroughly. For each of the three animals of the set 25 vessels were measured using every fourth glomerulus.

RESULTS

The general characteristics of the glomerulus of animals with additional pituitary tissue and those with none, as compared to the normal, can be well seen in microscopic sections. The glomeruli shown in Figure 1 are the ones used for making the models. The triple pituitary individual was a moderately affected case and was chosen so as to be representative. Others might have been chosen which would have been more extreme. The figure shows the typical simplification of the capillary tuft, and the reduction in size of capillaries, and the relatively great capsular space. The increase in prominence of what has been generally considered the basement membrane is shown. For the hypophysectomized animal the opposite condition is indicated, enlarged capillaries, thin basement membrane, and decreased volume of capsular space. A glomerulus of a relatively extreme case is shown in Figure 6. Here the increase in dark-staining material about the capillaries is marked. This is similar to the situation reported earlier.[5]

It is of interest that the change in what appears to be basement membrane also occurs in the pronephros of triple pituitary animals (Fig. 7). That this occurs early is evident.

The models of these representative renal corpuscles for one set of animals, made at a magnification of 2,000, show certain definite characteristics.[8] Relative glomerular size and its relationship to the capsular space are shown

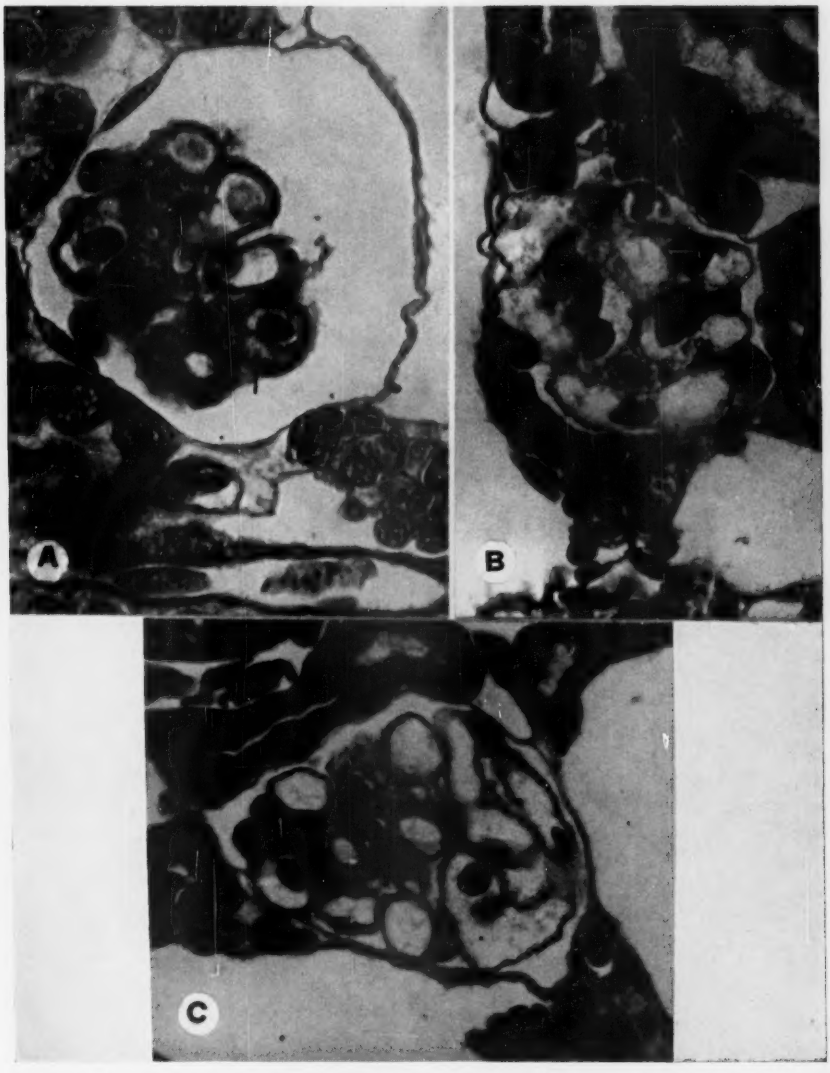


FIG. 1. Glomeruli from set of larvae used for models. Age 58 days. x700. A. Triple pituitary with two pituitary grafts. Had expanded melanophores, short gills and tail. Length 19.8 mm., arrow indicates basement membrane proper between epithelial and endothelial cells. B. Control, length 32.4 mm.; C. Hypophysectomized. Had contracted melanophores, long gills. Length 24.6 mm.

FIG. 2. Models of glomeruli within capsule. Relative size of glomerulus to capsular space. A. Triple pituitary; B. Control; C. Hypophysectomized.

FIG. 3. Models of glomerular surface showing clefts but no true lobation. A. Triple pituitary; B. Control; C. Hypophysectomized.

FIG. 4. Models of capillary tuft showing degree of complexity, capillary size and cross anastomoses. A. Triple pituitary; B. Control; C. Hypophysectomized.

FIG. 5. Cut models showing material between capillary lumen and capsular space, colored black. Shows capillary connections through the middle of glomerulus. A. Triple pituitary; B. Control; C. Hypophysectomized.

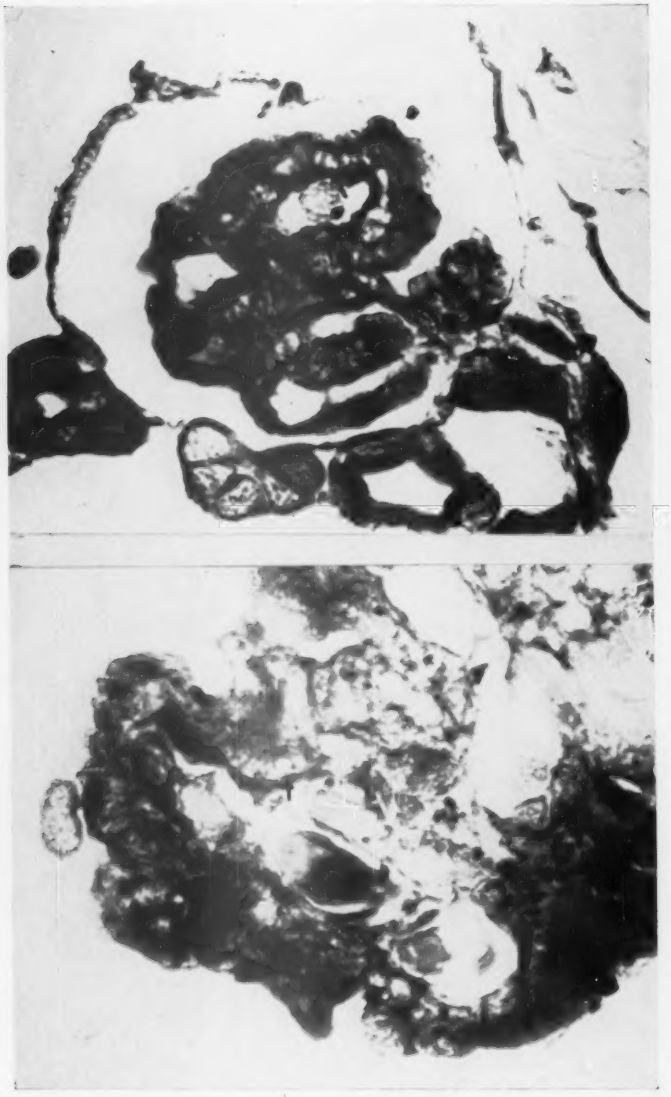


FIG. 6. Renal corpuscle of extreme triple pituitary animal. Thick basement membrane proper (arrow) external to which are clear spaces with occasional dots interpreted as foot processes of epithelial cells. x850.

FIG. 7. Pronephric glomerulus of triple pituitary animal. Capillaries with adjacent dark bands which may be basement membrane proper (arrow). Black dots with clear area about them thought to be foot processes. x900.

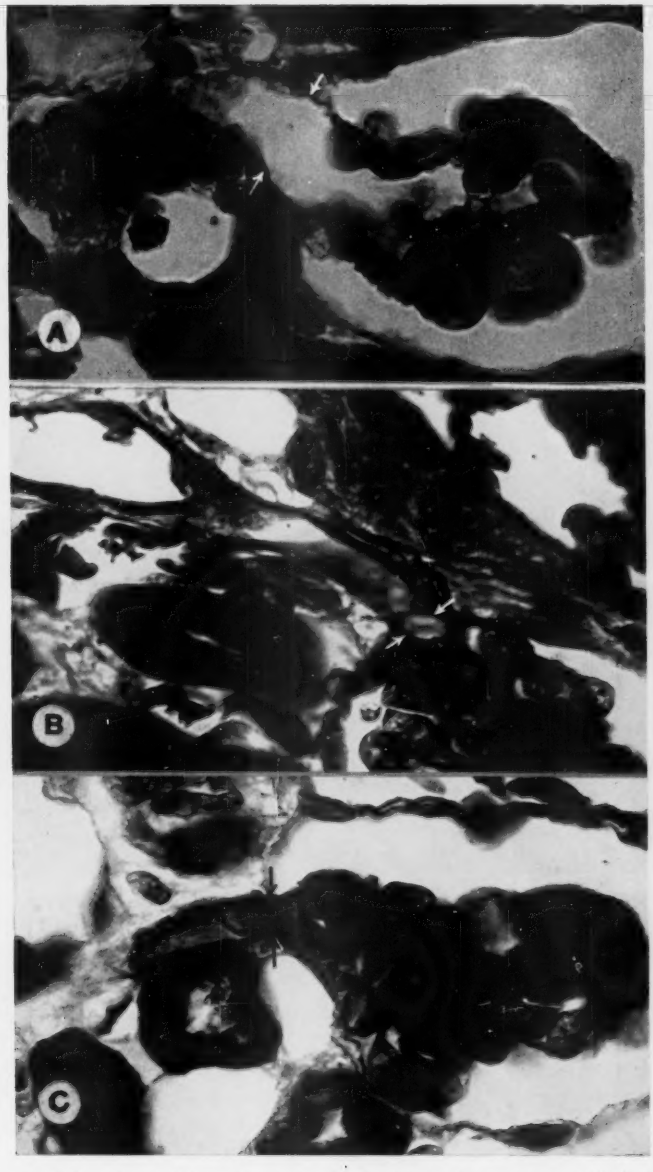
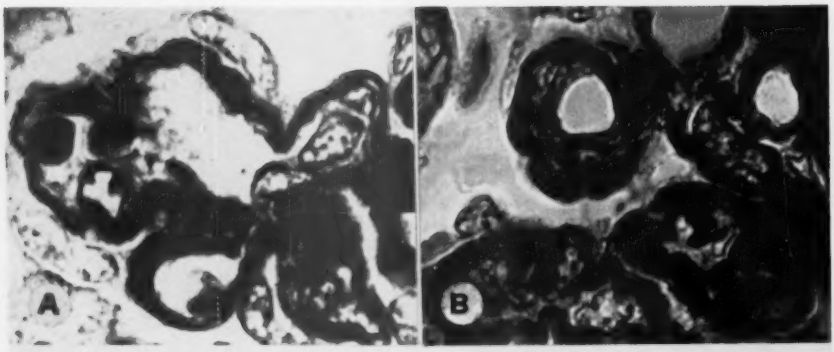


FIG. 8. Afferent arterioles of set of animals. Place of measurement indicated by arrows. x700. A. Triple pituitary, with enlarged arteriole; B. Control; C. Hypophysectomized showing only slight enlargement.



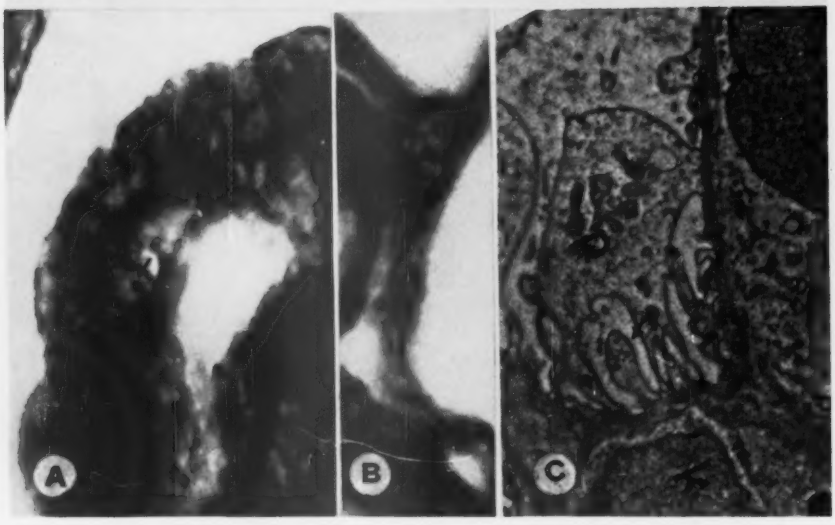


FIG. 9. Thickened basement membrane of human glomeruli under pathological conditions. Have been described as being porous. It is probable that these membranes include portions of the foot processes shown by electron microscopy. A. Essential hypertension, from McGregor, 1930. x1,500. B. Lipoid nephrosis, from Bell, 1950. x1,500.

FIG. 10. Comparison of the illustrations by the light microscope from our amphibian larvae with an electron micrograph. Arrows indicate a pedicel or foot process with surrounding space. A. Extreme triple pituitary animal. Portion of wall between capillary space and capsular space. x2,500; B. Control, x2,500. C. Electron micrograph of amphibian, from Pak Poy, 1957, x15,000.

in Figure 2. The animal with extra-pituitary tissue has a larger capsular space in respect to the glomerulus than does the normal animal, while that of the hypophysectomized animal is smaller although this is not so evident in the view of this figure. The percentages of the corpuscles represented by the glomerulus are approximately 50, 65, and 75 for the triple pituitary, control, and hypophysectomized animals respectively. However, the glomeruli for both of the test animals are actually smaller than for the normal and this is correlated with the smaller size of these individuals. Quantitative considerations have been discussed previously.[7] The elongated appearance of these corpuscles may be an artifact although they are often ovoid.

The surfaces of the glomeruli (Figs. 2 and 3) from two different views show the contour with protrusions of the nuclear areas of the epithelial cells. The surface is marked by clefts into the interior. These represent a larger proportion and are wider open in the triple pituitary animal. They are minimal in the hypophysectomized individual in which the glomerulus is relatively smooth. The surface modeling reflects the underlying capillary pattern (Fig. 4).

The models of the lumen of the capillary rete of the glomerulus (Fig. 4) reveal the afferent and efferent arterioles and their connecting vessels. The afferent arteriole widens after its entrance and before it divides to form the loops. This is particularly pronounced in extreme triple pituitary individuals. At points where the capillaries bend or anastomose. swellings are found. In addition to the principal loops, lying in the axis of the glomerulus, anastomoses become evident in all three types of animal. These are at various levels within the network. The differences in the capillary rete between the animal types are even more apparent than in the surface of the glomerulus. The small capillary tuft of the triple pituitary animal is made up of vessels with a diameter smaller than the control. There are ample spaces between these vessels which correspond to the clefts on the surface of the glomerulus (Fig. 3). In the hypophysectomized animal the whole tuft is smaller than in the control, but the diameter of the vessels is greater, with less free space between them as was reflected in the smoother external surface. The degree of complexity of the glomerular rete greatly varies. In the triple pituitary animal it is extremely simple, as can be seen in the model. This simplification is also true but to a lesser extent in the hypophysectomized individual.

When the whole corpuscle is cut through, as with the models in Figure 5, the thickness of the material between the lumen of the capillary net and the surface of the glomerulus is shown. This has been colored black. Without distinguishing them, it includes the endothelium, basement membrane, and the visceral layer of the epithelium lining the capsular space. It can be

noted that this thickness is greatly increased in the triple pituitary animal although the example chosen for making the model was not an extreme case. For the hypophysectomized animal this thickness is decreased. These differences will be made more clear by the description of the microscopic sections. The cut models also show the differences in the caliber of the vessels. The capillaries are seen to cross the interior of the glomerulus as well as appear on the periphery.

The afferent arterioles of representative glomeruli are shown in Figure 8. As reported earlier,[8] there is dilation of the afferent glomerular arteriole of the triple pituitary animals and to a lesser degree in the hypophysectomized individuals. The average diameter of the 25 measured (every fourth) for the triple pituitary is 12.1 micra and for the control 9.1 and the hypophysectomized 10.2, with the means 11.6, 8.6, and 9.7. The increase of the triple pituitary is significant in that there is a probability of but 1 in 3,000 that this is due to chance, while for the hypophysectomized individual it is 1 in 1,000. If all glomeruli were included, the largest measured for the triple pituitary was 21.3 micra and for the control 14.6.

Photomicrographs show that in the normal animal the distance across from the capillary lumen to the capsular space is about 1 micron (Fig. 1B). For the hypophysectomized animal this is less (C). In the triple pituitary animal it is greater (A) and may become as much as 8 micra (Fig. 6). That this is not all basement membrane proper is evident from the present studies. In Figure 1A, which is for a moderate case, it can be seen that there is cytoplasm of the epithelial cells external to the dark line of the basement membrane proper (arrow), and within this the cytoplasm of the endothelium. In this figure, at certain areas, there is the indication of fenestration and porosity. The glomerulus of an extreme case (Fig. 6) shows spaces which are probably for the pedicels or foot processes of the epithelial cells. These clear spaces are external to a thickened basement membrane proper (arrow) surrounding the capillary space. At greater magnification of such an extreme case the pedicels themselves are evident (Fig. 10A). These pedicels are seen throughout the territory external to the capillary and contribute markedly to the thickness of this area. By comparison (Fig. 10B) these structures can be identified in the normal animal at this magnification as dark spots surrounded by clear areas.

Similar changes occur in the basement membrane of the pronephric glomerulus of triple pituitary animals (Fig. 7). Here there is a similar thickening just external to the capillary space, which is interpreted as being the basement membrane proper (arrow), and there are numerous dots within clear spaces thought to be foot processes.

DISCUSSION

The mesonephric glomeruli of this study show anastomotic connections between the various capillary elements and thus agree with the work of Johnston[17] who made a wax model of a glomerulus of a human metanephros. The disagreement through the years as to these anastomoses has been finally settled in their favor by the work of Borst,[9] Hall,[14] and Boyer[10] as against Vimtrup,[24] Wilmer,[25] and others. Furthermore, many of these anastomoses can be seen in our models to pass through the center of the glomerulus and thus connect the principal elements that otherwise would form lobes. Thus our observations agree with those of Lewis[18] who points out that the rete does not form independent lobes as described by Hall[14] and Elias.[13]

We have found that the amount of body volume per glomerulus was greater in the larger animals, although there was no definite relationship between glomerular and body size. Since our triple pituitary animals are thought to be hypertensive, and show a large capsular space, it is of interest that McGregor[20] found this space increased in human hypertension. Distention of the capsular space has been correlated with physiological activity in amphibia by Hayman[15] and by Lucas and White.[19]

The dilation of the afferent arteriole in the triple pituitary animal is very definite, and it is interesting that such a dilation has been described in human hypertension by Jaffé[16] and others. It might be expected that extra tissue of the pars nervosa of the pituitary would bring about a contraction of this vessel, since the vessels of the gills and tail are thus affected.[4] Sawyer[23] has shown that posterior lobe hormones, particularly pitocin, cause contraction of the afferent arteriole in the frog. However, pitressin was found to be less effective, and its action on the glomerulus was said to be "merely a reflection of general vasoconstriction."

Earlier we described the basement membrane in extreme cases of triple pituitary animals, similar to the ones presented in the present paper, as seen by the light microscope.[5] Attention was called to the similarity in appearance to that described by McGregor[20] in human hypertension and by Bell[1, 2] in lipoid nephrosis as shown in Figures 9A and B respectively. That these figures, with the light microscope, include portions of the epithelial cells is indicated by electron microscopy and by a comparison with our figures for the triple pituitary animal. Electron microscopy of the kidney shows the basement membrane proper and that the epithelial cells have foot processes or pedicels lying upon its surface with spaces between them.[12, 14, 22] Hall speaks of the pedicels, lamina densa, and lamina fenestrata as functioning together as the "filtration apparatus" and also states that "the glomerular

basement membrane viewed with the light microscope must include to some variable degree all 3 structures." The wrinkling, porosity, and fenestration of the basement membrane in the above descriptions of pathology probably involve the foot processes or pedicels and the spaces between them. The cytoplasm of the epithelial cells in a zone adjacent to the basement membrane proper has greater electron density than the rest of the cytoplasm, and recently Farquhar, Vernier, and Good[18] have shown that in cases of lipoid nephrosis this is increased.

Pak Poy[21] studied these structures for the amphibian mesonephric glomerulus and shows that they differ from the mammalian metanephros only in being less complex. He states that the intricate pattern formed by interdigitation of the processes is lacking. A portion of one of his illustrations is shown in Figure 10C. In our preparations of extreme cases from triple pituitary animals the foot processes can be distinguished in the thickened area with the light microscope (Fig. 10A). In fact these can be made out in the glomerulus of the control animal (Fig. 10B). That this is normally within the powers of visibility with the light microscope can be realized when the magnifications are considered and these illustrations are compared with that of Pak Poy. It is believed that the greater part of this thickening is due to the enlargement of the pedicels.

The alterations in the pronephric glomerulus of the triple pituitary animal are similar to those in the mesonephros. If the dots within clear spaces external to the basement membrane proper are correctly interpreted as being pedicels, we believe that this is the first time these have been illustrated in the pronephros.

Among the multiple factors involved in the production of alterations in the glomerulus by additional pituitary tissue is the fact that the pars buccalis of the pituitary transplant causes an increase in the activity of the adrenal gland which may in turn be effective. In any case it seems certain that the pituitary is involved in some renovascular disturbances.

SUMMARY AND CONCLUSIONS

1. Development under the influence of additional transplanted pituitary tissue brings about many changes in the renal corpuscles of amphibian larvae, as does hypophysectomy.

2. Wax models of the corpuscle with its glomerulus present clearly further evidence of the anastomotic connections between the capillaries, and the cut models also show cross-connections through the center, indicating that the glomerulus is not truly divided into lobes but merely indented by clefts. The capillary tuft is shown to be simplified in "triple pituitary" ani-

mals and of reduced size. The simplification of the tuft in the hypophysectomized animal is associated with general vasodilation.

3. There is a significant dilation of the afferent arteriole in the animals with extra pituitary tissue. The slight increase in the diameter in hypophysectomized animals is not statistically significant.

4. What has been spoken of as the basement membrane, as seen by the light microscope, shows great changes. That of the hypophysectomized animal is thinner than normal. There is very great thickening of this material in an animal with extra pituitary tissue. Sections show this to include the foot processes of the epithelial cells as described with the electron microscope. These are of a size easily within the visibility of the light microscope in the extreme cases in which they are twice as large as normal. They can be by comparison identified even in the control. There is also a thickening of the basement membrane proper which would include the lamina densa.

5. Comparisons can be made between these changes in the amphibian mesonephros and the mammalian metanephros, particularly certain human pathological conditions. The present observations make it probable that what has been presented as basement membrane in microphotographs of these conditions includes portions of the epithelial cells.

6. It is believed that these changes are gradually brought about during development by long exposure to a continuous excess of pituitary hormones. This is indicated by the similar changes that are found in the pronephric glomerulus.

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EFFECTS OF X-IRRADIATION ON EMBRYOS AT CRITICAL STAGES OF HEART DEVELOPMENT‡

There is considerable evidence that differentiating cells are the most sensitive of all cells of the living organism. When embryos are x-irradiated at sublethal levels and subsequently studied histologically it is found that the regions showing the greatest radiation damage, as indicated by pycnotic nuclei, do not necessarily correspond with embryonic areas known to have a high mitotic index at the time of irradiation but rather with sites of active differentiation. The fact that the central nervous system has a protracted period of differentiation has been given as one of the reasons why it exhibits the most frequent and extensive damage following x-irradiation of embryos.[8]

Previous studies on embryonic and larval amphibians have indicated that cardiac muscle is relatively radioresistant.[7,9] However, these earlier studies did not deal with the effects of x-irradiation at specific stages of cardiac development. Therefore, the present study was undertaken to determine the effects of x-irradiating embryos at successive stages of cardiac differentiation.

MATERIALS AND METHODS

Embryos of Amblystoma punctatum were x-irradiated at Harrison's stages 26, 30, and 34 (Fig. 1). At stage 26, the primordial endocardial cells are separating from splanchnic mesoderm and are migrating toward the midventral region of the embryo, just caudal to the mandibular arch. By stage 30, the endocardial cells are grouped together, the bilateral layers of mesoderm have met in the midline, the splanchnic mesoderm of the cardiac region has thickened, and the future pericardial cavity is represented bilaterally as clefts between splanchnic and somatic mesoderm. By stage 34, the endocardial (endothelial) cells have become rearranged in a more definitive form giving a vascular lumen, and the primordial myocardium has extended completely around the endocardium in the cephalic regions of the heart. By this stage, the heart has attained its tubular form in the regions of the primordial conus, ventricle and

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atrium. The primordium of the sinus venosus is still bilateral and extends around the cephalic border of the liver primordium (Fig. 1). The first cardiac contractions begin at stage 34, when the myocardium is relatively undifferentiated. The presence of cross-striations in embryonic cardiac muscle at the time of beat initiation is questionable.[2, 8]

The embryos were exposed to single x-ray doses totalling 100r, 250r, 500r, 750r, 1000r, 1500r, 5000r, and 10,000r. The embryos were irradiated in circular celluloid dishes measuring 70 x 22 mm and containing approximately 20 cm^3 of spring water. The dishes were placed between two x-ray tubes providing vertical beams of radiation which traversed the water in opposite directions. Each x-ray tube was operated at 184 kvp and 30 ma., with 0.28 Cu and 0.50 Al filtration. The center of the dish was at a distance of 30.2 cm from the target of either tube for doses of 100r to 1000r, and at a distance of 21.5 cm for total doses ranging from 1500r to 10,000r. The dosage rate measured in air, at a point corresponding to the center of the water layer in the dish, was 250 r/min at the 30.2 cm distance and 500 r/min at 21.5 cm. It is unlikely that the differences in rate of exposure had any bearing on the results in these experiments since previous studies have shown that the significant factor in single dose exposures is the total radiation.

Following x-irradiation, the embryos were reared individually in Syracuse dishes containing approximately 20 cc of Great Bear Spring Water. The dishes were kept on stone top tables provided with running tap water and the temperature of the spring water within the dishes ranged from 12 to 13° C. The dishes of control and experimental animals were kept side by side and under the same conditions.

The embryos were studied at regular intervals under a binocular dissecting microscope and data were recorded on the rate of differentiation, on the number and type of malformations, and on the presence of cardiac contractions and circulation. Representative cases were fixed at various postirradiation periods in Bouin's fluid, sectioned at 10 μ and stained in hematoxylin and eosin. Other fixatives have been used for cytological studies which are in progress.

OBSERVATIONS

1. *Effects on rate of differentiation and on survival.* Harrison's stages of normal development of Amblystoma punctatum have been adopted by practically all embryologists as a method of recording steps of differentiation in Amblystoma from the fertilized ovum (stage 1) to a swimming larva which has resorbed most of its yolk and begun to feed (stage 46).

Some of the stages pertinent to heart development are shown in Figure 1. Other stages of particular importance in the present study include stage 36 when circulation is initiated, stage 39 when the sinus venosus becomes established as the cardiac pacemaker, and stages 42 to 45 when the heart attains a form approximating that seen in the adult salamander.

It should be noted that various stages of differentiation proceed at unequal rates. For example, development from stage 20 to 35 will occur in a 4-day period in normal embryos reared at a temperature of 12 to 13°C, whereas development from stage 40 to 46 covers a span of about 16 days at the same

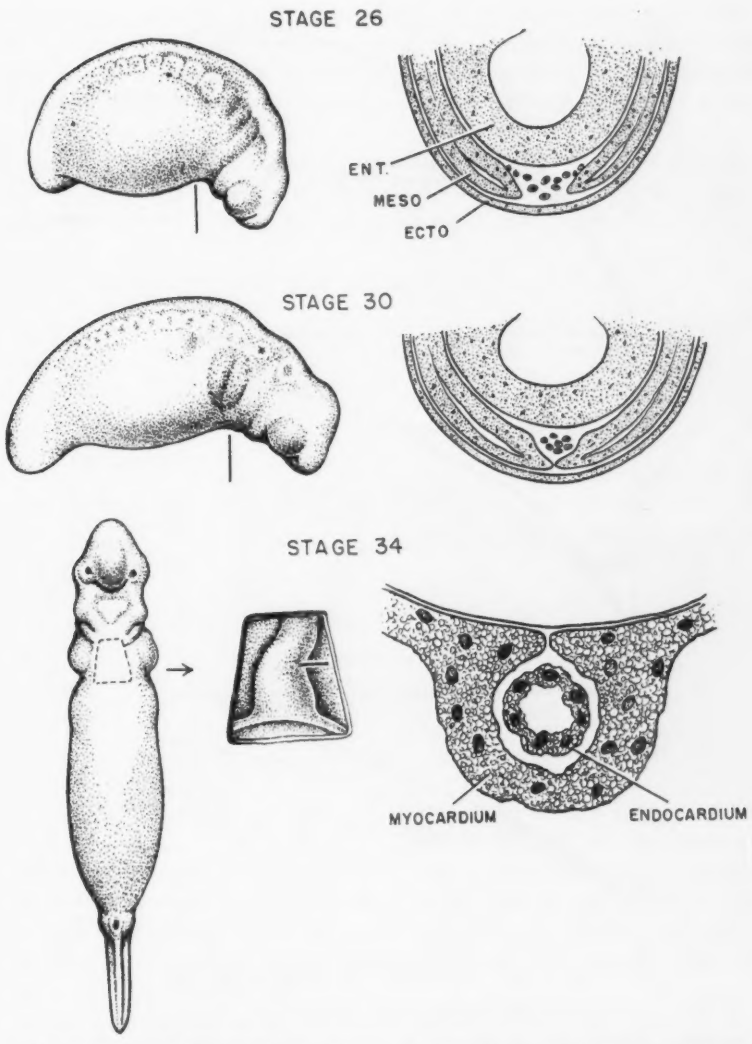


FIG. 1. Cardiac differentiation in embryos of Amblystoma punctatum at Harrison's developmental stages selected for x-irradiation. Vertical lines beneath the stage 26 and stage 30 embryos indicate the plane of the cross sections shown at the right. The embryos are at x12; the sections of stages 26 and 30 are at x38; the stage 34 heart exposed by opening the pericardial cavity is at x30, and its cross section is at x150.

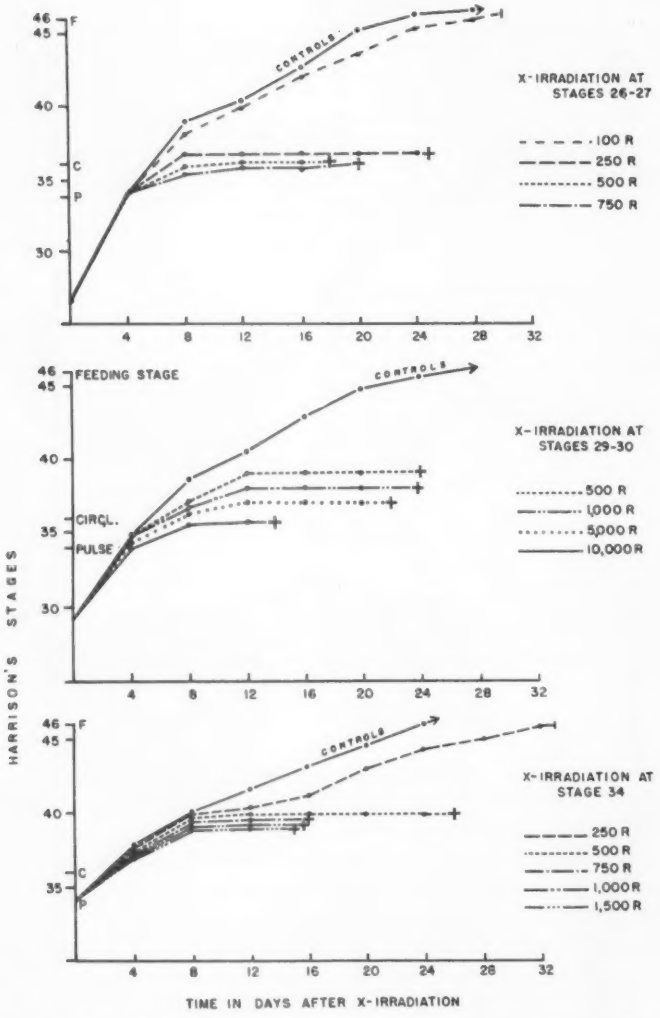


FIG. 2. Graphs showing the amount of development (expressed by Harrison's stages) at 4-day intervals after x-irradiation. Arrows at end of control lines indicate continued normal development; vertical lines at ends of two curves for irradiated series show that the animals failed to feed and ceased development; crosses at end of curves for other irradiated series indicate death of all animals in the series. Each curve represents data for 15 embryos.

temperature (Fig. 2). Furthermore, it must be emphasized that the developmental rate varies with temperature and that comparisons of normal and experimental embryos, and of different sets of experiments, cannot be made on a time basis as shown in Figure 2 unless all embryos are reared at the same temperature.

As shown in Figure 2, the x-irradiated animals developed at approximately the same rate as the controls for 4 days, thereafter began to show more retardation by 8 days, and frequently ceased differentiation by 12 days.

TABLE 1. EFFECTS OF EXPOSURE TO A SINGLE DOSE OF 500R X-IRRADIATION

Stage at time of radiation	*Time in days after radiation*					
	4	*8*	*12*	*16*	*20*	*24*
	Percentage of embryos surviving					
26	100	80	60	0		
30	100	100	100	100	86.6	0
34	100	100	100	100	90	0
	Percentage with eye and CNS abnormalities					
26	0	100				
30	0	100				
34	0	100				
	Percentage with cardiovascular defects					
26	0	20	100			
30	0	0	80	100		
34	0	0	100			

In general, the amount of developmental retardation was proportional to the total amount of x-irradiation and inversely proportional to the degree of differentiation at the time of irradiation, i.e., radiation at stage 34 had less effect than at stage 26. For example, embryos given a single dose of 250r at stage 26 stopped development at stage 36, whereas embryos given 250r at stage 34 continued to develop to stage 46 but since they failed to eat they never developed beyond stage 46.

The graphs of Figure 2 show the time when no survivors remained in a series. However, since each curve represents data for 15 embryos and since individuals died at various intervals after x-irradiation, further details are necessary to accurately portray survival time. Table 1 presents data on percentage of survivals at 4-day intervals following a single dose of 500r. At 12 days, for example, the number of survivals after irradiation at stage

26 was only 60 per cent (or 9 embryos out of 15), whereas the survivals for embryos irradiated at stages 30 and 34 were 100 per cent.

2. *Abnormalities of central nervous system and eyes.* As shown in Table 1, none of the animals exposed to a single dose of 500r at the selected stages showed any head and eye abnormalities which could be observed in the intact animals under the dissecting microscope at 4 days after irradiation although 100 per cent exhibited such abnormalities by 8 days. On the other hand, embryos exposed to 5,000r frequently showed retarded development of the head and eyes by two days after radiation.

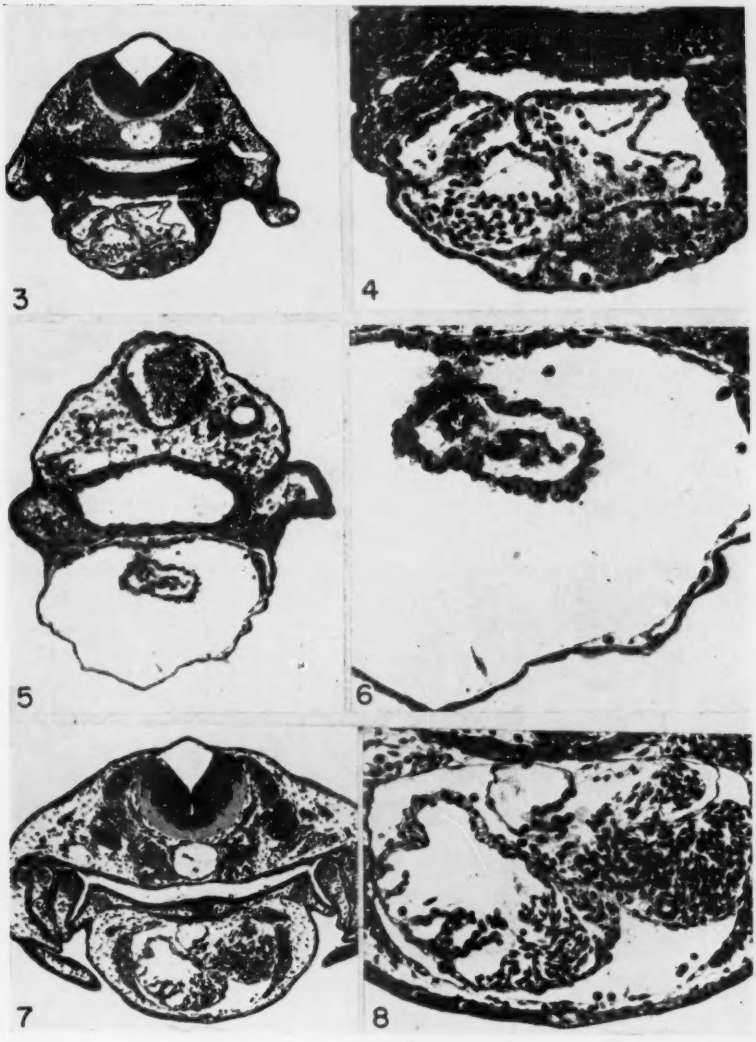
The most common abnormalities observed in the early postirradiation periods were microcephaly and small eyes. Eventually, there was extensive necrosis of most of the brain and spinal cord in all embryos exposed to 500r or more at stage 34, and in all animals exposed to 250r or more at stage 26. Photomicrographs of sections of typical cases are shown in Figures 5, 9, and 11.

3. *Abnormalities of cardiovascular system.* Considerable data on the circulatory system can be obtained by observing blood flow in the vessels of the gills, liver, and yolk, and the heart itself can be studied through the translucent ventral body wall. In this way one can determine whether pulsations begin at stage 34, whether circulation begins at stage 36, and whether the contraction rate is normal. Since radiation retards growth and differentiation in general, one must compare the experimental animals with normals of a similar Harrison developmental stage as well as with the simultaneous nonirradiated controls of the same age.

The cardiovascular effects of exposing embryos at the selected stages to a single dose of 500r are shown in Table 1. When the embryos were irradiated at stage 26, circulatory disturbances were noted in 20 per cent of the animals by 8 days and in 100 per cent by 12 days. The abnormalities appeared somewhat more slowly in embryos exposed at stages 30 and 34.

The most commonly observed effects included stasis in vessels of the yolk, deficient gill circulation, and failure of the heart to empty completely at systole. It was striking that the rate of contraction was often approximately normal for a considerable period after the heart showed marked reduction in amplitude of contraction.

Reference to Figure 2 shows that embryos given 100r at stage 26 develop to stage 46 at a rate only slightly below normal and heart development appears normal. Exposure to 250r at stage 26 produces a more marked retardation in development, but all cases of this series developed circulation which appeared normal for a period of time and then became deficient later. Exposure to 500r at stage 26 permitted general body development to stage



FIGS. 3-8.

Photomicrographs of sections of control and irradiated embryos.

FIG. 3. Section of a normal animal at stage 37 showing the CNS dorsally and the heart ventrally, at x32.

FIG. 4. Shows the stage 37 heart at higher magnification, x80.

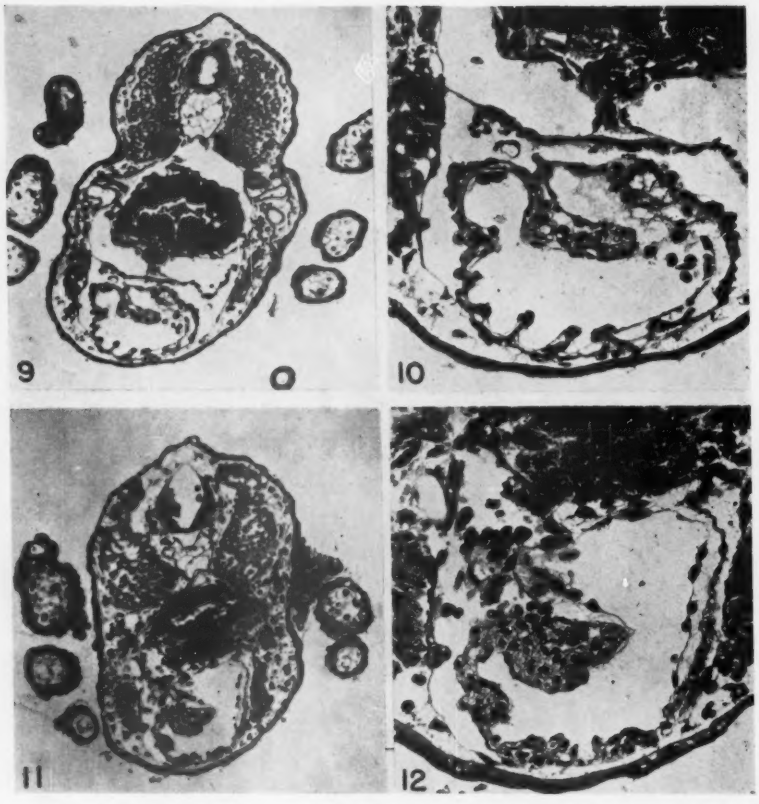
FIG. 5 is from an embryo fixed 9 days after 10,000r at stages 29-30. Note small heart, enlarged pericardial cavity, and necrosis of CNS, x32.

FIG. 6 shows the heart from Fig. 5 at higher magnification, x80.

FIG. 7 is from a normal animal of stage 41, x32.

FIG. 8 shows the heart from Fig. 7 at higher magnification, x80.

The irradiated animal used for Figs. 5 and 6 approximated a stage 37 (Figs. 3 and 4) as regards balancers and body development, but its gills were only differentiated to stage 36, and the heart was an abnormal stages 34-35. The nonirradaited controls of the same age were at stage 40, only slightly less differentiated than the normal shown in Fig. 7.



FIGS. 9-12.

Photomicrographs of embryos x-irradiated at stage 29-30 with intermediate doses.

FIG. 9 is from an embryo fixed 16 days after exposure to 500r. The embryo was at stage 38-39 at time of fixation. Note retarded CNS, enlarged neural canal and scarcity of corpuscles in heart, x32.

FIG. 10 shows the heart from Fig. 9 at a higher magnification, x80.

FIG. 11 is from an embryo fixed 16 days after exposure to 1000r. The embryo had reached stage 38 when it was fixed. Note necrosis of CNS and complete lack of blood corpuscles in heart, x32.

FIG. 12 shows the heart from Fig. 11 at a higher magnification, x80.

The nonirradiated controls of the same age were at stage 42, or slightly beyond the normal shown in Fig. 7.

36, the circulation stage, but circulation never developed in more than 80 per cent of the series shown in Table 1. In a more recent study, including more cases, it has been found that circulation never develops in more than about 60 per cent after exposure to 500r at stage 26. On the other hand, embryos given 500r at stages 29 and 34 regularly develop circulation of apparently normal type for a considerable period before exhibiting cardiovascular abnormalities.

Exposure to 10,000r at stages 29-30 regularly stops development at stage 35 plus, before circulation normally begins. Examination of sections shows that the heart is markedly retarded and is even smaller than in normal animals of comparable developmental stage as regards differentiation of gills and general body characteristics. Several degrees of cardiac abnormalities are illustrated in Figures 6, 10, and 12.

4. *Other Abnormalities.* Abnormal curvature of the spine was a common postirradiation effect. Many of the embryos exposed at stages 26-27 developed kyphosis by stages 35-36. Kyphosis was less frequent after irradiation at stages 30 and 34. On the contrary, many of the embryos exposed at the latter stages developed with an abnormal backward curvature of the tail and with some lordosis of the body.

The irradiated embryos frequently appeared more pale than their controls. A sparseness of pigment was noted particularly on the head and eyes.

Localized swellings occurred frequently, particularly over the region of the heart and liver. Figure 6 shows an example of an enlarged or swollen pericardial cavity and a small, retarded heart in a section of an embryo fixed at stage 36, and at 9 days after a single exposure of 10,000r at stage 30. A question arises whether the enlargement in this case is a type of edema which is related to retarded cardiac development and lack of blood circulation. However, the condition in the case illustrated has occurred at a stage when circulation in controls has just begun. Furthermore, there have been numerous cases when large swellings have appeared even before the time when circulation normally begins. It seems clear that the relatively large localized swellings differ from the generalized type of edema which appears over most of the body of animals which survive in the face of a circulatory abnormality for long periods beyond the time when circulation is normally initiated.

DISCUSSION

It has been noted that the experimental and control animals were reared at the same temperature. This is important because: (i) the rate of development of amphibian embryos varies with temperature and (ii) the speed at

which postirradiation effects appear varies with temperature. Allen, Schjeide, and Hochwald[1] showed that the temperature of tadpoles during x-irradiation makes little difference in the ensuing processes, but that low temperature subsequent to irradiation delays the time when the destructive processes appear. In the present study, it has been shown that embryos exposed to 500r at stage 30 and reared subsequently at a temperature of 12 to 13°C develop to Harrison's stage 39 by 12 days and then come to an approximate halt in differentiation even though some embryos survive for 24 days. There is no doubt that most of the destructive processes would have appeared sooner and the survival period would have been shorter if the embryos had been reared at a higher temperature. However, we need more studies to determine whether the plateau in differentiation appears at the same stage at different temperatures. For example, we do not know whether the plateau in differentiation for embryos irradiated at stages 29-30 will occur consistently at stage 39 after 500r, at stage 38 after 1,000r, and at stage 37 after 5,000r regardless of postirradiation temperature.

The question of localized swellings on irradiated embryos has been discussed with the observations. It has been noted that the swollen regions ventral to the heart and liver differ from generalized edema observed in accompaniment with circulatory disturbances following other types of experiments on amphibian embryos. There is a good possibility that part of the effect is due to damage to the pronephros and to lack of excretion, as suggested in other studies.[8] However, it is doubtful whether this completely explains the enormous amount of swelling which sometimes occurs relatively early.

The present studies are in keeping with previous studies in showing that x-irradiation of embryos damages the developing central nervous system early and consistently. On the other hand, damage to the heart and circulation was found more consistently than in previous studies of x-irradiated amphibian embryos.[9] It is felt that this is correlated with the fact that the embryos were exposed at critical stages of heart differentiation and they were examined with particular reference to cardiovascular damage.

In comparing our results on amphibian embryos with those described by Liljestrand and Edwards[4] for offspring of x-irradiated pregnant rats, one must consider differences in the stages studied as well as species differences. They did not find cardiac malformations in offspring which had reached 10 days of age but, as they themselves point out, this does not exclude the possibility that cardiac malformations were present in fetuses which died before birth. In the present study on amphibian embryos, the cardiovascular defects were found concurrently with other lesions and none of the embryos

survived to a stage of differentiation which correspond with that of a newborn rat.

A question arises as to how much of the circulatory damage results from radiation of cardiac tissue and how much is dependent on damage to other parts of the embryo. Some of the experiments show retardation in heart development and weak cardiac contractions at a time when the embryo exhibits a fairly good functional development of the nervous system as indicated by its response to tactile stimulation. Many cases never develop any blood circulation even though the gills and a number of other organ systems differentiate to a degree characteristic of embryos at the time when circulation normally begins. On the other hand, the results show that some of the radiation dosages at the selected stages are followed by the development of a normal blood circulation for several days and then circulation gradually fails concurrently with an increase in other malformations. Experiments on the eye[5] and on the ear, medulla, and forelimbs[6] show that organ primordia transplanted from irradiated to normal animals generally attain a higher degree of differentiation than under the conditions of total-body irradiation. This suggests that heart primordia transplanted from irradiated animals to normals might have their developmental and functional period prolonged. It is conceivable that an irradiated heart which is destined to die under the conditions of total-body irradiation might survive and function indefinitely after transplantation to a normal animal. Experiments of this type are in progress in order to gain more information on how much of the cardiac damage is due directly to radiation of the cardiac primordium.

SUMMARY

1. Embryos of Amblystoma punctatum were x-irradiated at Harrison's stages 26, 30, and 34. These are critical stages of heart differentiation ranging from the time when the primordial endocardial cells separate from splanchnic mesoderm to the time when the heart attains a tubular form and begins to contract.

2. The x-ray exposures ranged from 100 to 10,000r. Following x-irradiation, the embryos were reared at a temperature of 12 to 13°C. Non-irradiated controls were kept at the same temperature.

3. In general, the amount of developmental retardation was proportional to the total amount of x-irradiation and inversely proportional to the degree of differentiation at the time of exposure.

4. In all of the series studied, there was a latent period between exposure to x-irradiation and the appearance of developmental defects.

5. The embryonic central nervous system and eyes were particularly radiosensitive. Microcephaly developed in a high percentage of embryos exposed at the early experimental stages (26 and 30).

6. Cardiovascular anomalies developed in all embryos exposed to 250r or above at stage 26, and in all embryos exposed to 500r or above at stages 30 and 34. With high doses, differentiation was so retarded that the embryos never developed circulation. With intermediate doses, circulation developed for a period, only to fail subsequently. The pulsation rate remained approximately normal for a considerable period after the magnitude of contraction had decreased. Stasis in vessels of the liver and yolk was common.

7. Since the heart anomalies occurred together with other malformations, further studies are in progress to determine how much abnormal cardiac structure and function results directly from radiation of the embryonic heart and how much is dependent on total-body x-irradiation.

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DEVELOPMENT REACTION TO ENVIRONMENT**

Every organism, including man, lives under the influence of not one, but two environments. The one more generally recognized is that which surrounds it, hence is external. However, each organism also has an internal environment set up by its internal organs, their function and the secretions, hormones, enzymes, etc., which they produce.

Every organism, from the simplest to the most complex, responds to the external environment, largely by neuromuscular activity. This response constitutes its visible or overt behavior. However, the responses made to the internal environment, which constitute the invisible or covert behavior of the organism, have a profound effect in modifying overt behavior, and vice versa. Hence, the over-all response exhibited by an organism is a resultant of the interaction of the responses to both environments and varies greatly in its specific nature, depending on a variety of factors set up by its immediate needs and capacities.

Whatever the nature of the response may be, it is a reaction, involving activation and inhibition, of the whole organism capable of functioning, not of any specific part alone. This is obvious in the normal adult. In the developing organism it is not so generally recognized, but is, nevertheless, equally true. Because the various organs, including the nervous system and the muscles, develop in a seriatim manner, they do not all reach a functional state at the same time. Consequently, the behavior of an embryo or fetus is constantly changing as new neural connections, both within and without the central nervous system, and new organs attain a functional state. It is to the earlier aspects of this changing pattern that this communication is directed.

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Many in previous centuries have written down their observations of embryonic movements in many animal forms,[16, 18, 20, 48] but these were more or less casual observations. It is only within the last 50 years that any real attempt has been made to study carefully the sequence of development of the neuromuscular apparatus in relation to behavior. The work of Coghill, summarized in his book,[13] which was not only the first but the most complete physiological and morphological study of any form to the present, established the nature of the development of the amphibian, Amblystoma. In essence, his conclusions indicate that, when response to the external environment begins (specifically to exteroceptive stimulation with a fine hair), all of the embryo capable of neuromuscular function participates in the reaction. As the neuromuscular mechanism continues to develop, which it does in a cervico-caudal direction, the response pattern expands caudally.

Because of the involvement of all of the functional neuromuscular mechanism in this expanding response, Coghill termed this type of reaction a "total pattern," i.e., a participation of the whole organism capable of functioning in such an activity. As he states,[13] "The behavior of this animal [Amblystoma] begins as a total reaction, and continues as such until after locomotion is established" (p. 125). Later, when still further neural development makes them possible, specific reflexes appear. These Coghill termed "partial patterns" and regarded them as being "individuated" from the total pattern, though the latter always remains dominant.

The early work of Swenson (unpublished) and all of Angulo's observations[2-5] (both of them students of Coghill's at Kansas who went with him to the Wistar Institute) convinced Coghill that the basic principle of the developmental sequence of behavior established for Amblystoma held also for the rat and probably was of universal application to all vertebrates, including man. His 1929 paper,[14] setting forth this belief for the human embryo, was not too well received. However, this application of the basic principle derived from Amblystoma to man was no purely theoretical extrapolation, as was assumed by some. It was based, not only on the then incomplete work on the rat, to which reference has already been made, but also on data derived from publications of Erbkam,[15] Zuntz,[62] Strassmann,[46] Yanase,[60] Krabbe,[33] Bolaffio and Artom,[10] and M. Minkowski.[30-39] Unfortunately for Coghill's purposes, only the papers by Minkowski have any validity as far as the recorded observations are concerned. Many of the others contain only cursory observations and all of the others were carried out under conditions so abnormal as to render the results suspect.

Most students in the field have accepted Coghill's conclusions for Amblystoma, even when, with varying degrees of dissent, they have objected to the application of his basic principle to other vertebrates and, especially,

to man. This is the more interesting as it is well known that the development of the neural tube and of the musculature is identical in principle in all vertebrates. Consequently, one might expect, a priori, that functional development might also follow a common principle. However, two basically different schools of thought arose, one following Coghill's principles, one diametrically opposed to them. In addition, several minor variations of less importance have arisen.

The major hypothesis opposing Coghill's principles, although originally put forward by Swenson[47, 48] in his later papers, has been fostered largely by Windle and his students.[58] This group has postulated, especially for mammals and man, that behavior develops by the appearance of a 'simple' reflex, usually in the head or upper extremity, then of another which is integrated with the first, of a third and so on. In other words, the individual 'simple' reflexes are the building blocks which, secondarily integrated, form behavior.

The concept of the 'simple' reflex undoubtedly comes from Sherrington,[44] who used the term as "probably a purely abstract conception" but found it a "convenient, if not a probable, fiction." When integration occurs is a matter quite aside from the probably fictitious nature of simple reflexes. On this score, Coghill[18] had no doubts as to Amblystoma. There integration is always primary, never secondary. Integration must occur before a reflex becomes elicitable. Each alteration in response pattern is preceded by the development of the specific neural connections needed for its execution and their integration with existing neural mechanisms. Then only can the new addition become functional. This is also the generally accepted view of most neurologists[37] for nervous system integration in all vertebrates.

A number of investigators have concluded from their studies that the basic principle of 'total pattern—partial pattern' set forth by Coghill holds good in at least some species in nearly all of the vertebrate classes. Among these are Tracy,[50] on Opsanus, Coghill, himself, for Opsanus and Fundulus,* Youngstrom[61] on Rana, Acris, and Pseudacris, Wang and Lu[58] on Rana and Bufo, Tuge[51] on Terrapene, Smith and Daniel[45] on Caretta, Tuge[52, 53] on pigeons, Kuo[34, 35] on the chick, Angulo[3, 4, 5] on the rat, Pankratz[40] on the rabbit, Barcroft and Barron[7, 8] and Barron[9] on sheep, and the Pittsburgh studies[21–32] on man.

It is important to emphasize that it is the basic principle of Coghill's concept of 'total pattern—partial pattern' that these investigators agree upon as having a wide application. Each form studied has its own minor variations, even in the early stages. As the embryos of the different classes

* See Herrick,[19] pp. 96-97, 94-96, and 264-267 for a posthumous account of Coghill's work on these forms.

grow older, more marked variations occur, until major differences become evident. These are the characteristics of the species being studied, for variations exist even in the same family. Of course, as the organism under the study rises in the phylogenetic scale, the more specialized it becomes and the greater is the change in its behavioral capacities. It should also be noted that there are very minor differences even in the same species, hence the emphasis on the basic principle.

When Coghill first suggested the universal application of his Amblystoma pattern of behavior, there was relatively little evidence, confirmatory or otherwise, upon which to base his idea insofar as man was concerned. Although it had long been this writer's desire to study such living human fetuses as might become available from time to time, it was not until the autumn of 1932 that it became possible to do so. When it did become possible to make such observations, it seemed advisable, *inter alia*, to check on Coghill's hypothesis regarding man's earliest developmental activity sequence.

Nearly 28 years and more than 150 cases later, the sequential pattern of the different stages forming the fetal components of human overt behavior is beginning to assume a recognizable form, although much still remains to be done. From the evidence now available, it would appear that, as human overt behavior develops, a number of different major categories of activity are manifested. The first two to appear consist of exteroceptively elicitable reflexes and proprioceptive reflexes resulting from muscle stretch and other appropriate stimuli. A third early type of movements to be exhibited are of the kind generally denominated as spontaneous. Voluntary movements, almost certainly not found in fetal life, are the last to appear and eventually constitute the major part of the motor activity of the adult. Each major type of activity, with the possible exception of proprioceptive movements, passes through a distinctive development of its own. However, almost none of the essential elements of any of these categories of activity is ever lost. Rather, the earlier forms of activity are gradually incorporated, with a new orientation, into the overt behavior pattern characteristic of any particular age of the organism. It is true, however, that the earlier types of activity usually assume less important roles as later types take over.

The exteroceptive reflex category of activity begins at about 7½ weeks of menstrual age.* At the moment, it is not possible to state definitely when proprioceptive activity begins. It almost certainly begins as soon as the exteroceptive reflex type.[20,58] So-called spontaneous movements begin very

* All age references given will be to the menstrual age of the human fetus.

shortly after the exteroceptive reflexes, by 9½ weeks, if not before, but do not assume any great importance until the 18th week or later. These constitute the overt behavior of the fetus and neonate. As noted, voluntary activity almost certainly does not make its appearance until later in infancy.

From 7½ weeks to about 9½ weeks, the progressive development of the reflexes is dependent upon the level of development of both the nervous and muscular systems. Thereafter, the changes depend almost entirely upon the developmental level of the nervous system alone, as practically all muscles necessary for movements have appeared by that time. Just as little or nothing of the activity patterns is ever lost, so little or nothing of the underlying nervous mechanisms that makes them possible disappears. On the contrary as the fetus develops, the neural mechanisms previously present become incorporated into the expanding mechanism of the late fetus, the infant, and the adult.

At 7½ weeks, the typical reflex is elicitable on stimulation with a fine hair in the sensitive area located about the mouth and the alae of the nose. This is the region supplied by the maxillary and mandibular divisions of the trigeminal nerve. The sensitive region at this age is, however, much more restricted than is the case later. No other region of the fetal integument is reflexogenous to light stimulation at this age. However, both at this age and for a brief period before it, as well as thereafter, movements may be secured by direct mechanical stimulation (pressure or pricking) of the musculature or by its electrical stimulation. Such activity, of course, is not reflex.

At 7½ weeks, the typical reflex in response to stimulation with a fine hair in the reflexogenous area is a lateral flexion of the neck away from the side stimulated. By 8 weeks, the area exhibiting predominantly contralateral flexion has begun to expand caudally to include the uppermost trunk and shoulder muscles, the latter feebly. By 8½ weeks, the entire trunk enters into the chiefly contralateral flexion, both brachia extend markedly at the shoulders, and the pelvis shows some rotation. These activities become more complete by 9½ weeks. During this period, all movements of the upper and lower extremities are brought about by their girdle muscles and are exhibited only in conjunction with trunk movements. Neither upper nor lower extremities are capable of independent movement until later.

During the two-week period from 7½ to 9½ weeks, the reflexogenous area of integument supplied by the maxillary-mandibular divisions of the trigeminal has expanded slightly. By 10 to 10½ weeks, the upper eyelid has become sensitive to light stimulation, but until 10½ weeks no other area of integument over the entire body is reflexogenous. At 9½ weeks,

local reflexes appear in the face area, at 10½ weeks in the upper extremity, at 11 weeks* in the lower extremity, and at 13 weeks in the trunk.

The responses of the human fetus between their first appearance at about 7½ weeks and the beginning of local face reflexes at 9½ weeks constitute a total pattern in Coghill's sense. From the beginning they involve all of the neuromuscular mechanism capable of functioning reflexly in the entire organism. At their appearance they are highly localized in the neck region, but as new neural and nerve-muscle connections are established they expand caudally. As these new connections within and without the nervous system develop, they are primarily integrated with those already existing. Specific local reflexes begin to appear or, to use Coghill's terminology, partial patterns begin to be individuated from the total pattern at 9½ weeks and continue to be elicitable in a caudal direction. By 14 to 14½ weeks, or earlier in some individuals, the total pattern has largely been replaced by specific local reflexes (the partial patterns of Coghill), but the total pattern never entirely disappears.

At about 15 to 16 weeks, the fetus seems to become relatively inert. Certain reflexes, as those in response to stimulation of the palm and sole, may be elicited until stopped by hypoxia, but many others become very difficult to evoke. Curiously, though this same phenomenon was noted by Barcroft and Barron[7,8] in the sheep fetus, it has not been mentioned by those who studied other mammals. This sluggishness of response persists until about 18 to 20 weeks, though a vestige of it remains until ages at which the fetus may be resuscitated. However, lack of oxygen does not appear to be the primary cause of the sluggishness observed.

The fetus may be temporarily resuscitated at about 22 weeks or a little earlier. When resuscitated, the fetus becomes very active. From this time on until birth, the great difficulty in studying reflex responses is to distinguish between movements evoked by the stimulus applied and activities of the so-called spontaneous category that might have appeared in any case. From 22 weeks to birth, spontaneous activity becomes the chief type of movement exhibited.

The so-called spontaneous category of fetal activity makes its appearance by 9½, possibly, though very rarely, by 8½ weeks of age. At the time of its first appearance, it plays a very minor role. This role increases with the age of the fetus, assuming some importance by 18 weeks and becoming the dominant type of activity in later fetal life, as already noted. When it first appears, reflex activity is in the phase of neck-trunk total pattern move-

* In earlier publications, this date was given as a few days earlier. A careful recheck of all protocols and motion picture records has proved the earlier age to be in error.

ments. The spontaneous activity exhibited at this age consists of short bursts of side-to-side repetitions of the same kind of movements elicitable reflexly. In general, spontaneous activity seems to lag slightly behind reflex capacity in the appearance of the different types of movement.

Much has been presented[19, 54, 55] on spontaneous activity with little illumination as to its precise nature and cause or causes. Some observers, like the writer[30] in earlier communications, have believed some spontaneous movements to be reflexes in response to unrecognized exteroceptive stimuli. In the Pittsburgh collection of motion pictures of living human fetuses is one of an 8½ weeks' case (#116) which responded to each of two widely separated stimulations with a fine hair in the side-to-side manner characteristic of spontaneous activity. This is the only case in which a known stimulus, in so young a fetus, has been seen in the Pittsburgh studies to cause such activity. However, at 9½ weeks and later, the handling during extraction of a fetus has frequently brought on the spontaneous type of movement. This is probably also elicited by the stimulus of handling. In the human fetus, contrary to the author's findings in the rat fetus (unpublished), spontaneous movements are of relatively short duration until 20 to 22 weeks, when some resuscitation may be possible.

Paton[41, 42] and Wintrebert,[59] among others, have demonstrated an intermittent rhythmic automatism of the trunk musculature in selachians. Such movements are clearly indicated by Wintrebert to be myogenic in character. Paton, however, believed the similar movements he observed were due to the presence of nerve-impulse-transmitting intercellular bridges, shown by Harrison[17] not to exist. Wintrebert[59] thought these early myogenic activities—they precede reflex movements—might aid the respiration of the embryo. Tracy[49] is of the opinion that the earliest movements of the toadfish, Opsanus tau, are caused by internal stimulation by the accumulation of CO_2. There is clear evidence from a number of sources that such O_2-CO_2 imbalance may cause muscle contractions in fishes prior to the onset of reflex activity. Angulo[2] refers to "endogenous stimulation by metabolites," meaning CO_2, as a cause of spontaneous movements in albino rat fetuses. Graham Brown[16] pointed out that progression in adult decerebrate cats may still occur rhythmically after deafferenting the hind limbs. He believed that the efferent neurons acted in such cases as "half centers," stimulated from within by CO_2. They could also be activated by balanced alternate stimulation and inhibition from cephalic levels of the nervous system.

Weiss[57] has demonstrated that, under experimental conditions, the central nervous system may give rise to spontaneous activity through automatically released rhythmic bursts of impulses to muscle tissue. Such discharges have

been found in the respiratory center,[1, 11] where CO_2 is the stimulus. However, automaticity may be an inherent property of the central nervous system without such stimuli.

Certainly, spontaneous activity is nonvoluntary in character, whatever its actual cause may be. At this time, it appears likely that there is no one cause, but that a variety of causations produce the movements termed spontaneous. Hence, it may be that spontaneous movements are, in some cases, responses to the external environment, in others to the internal environment, or even to both, or possibly to neither. In the last case, they may be truly automatic.[11]

Under normal circumstances, the mammalian fetus *in utero* has a quite constant external environment. There is, of course, no method of determining whether the very early types of activity occur in this constant external environment, before the so-called 'quickening.' However, there is no question of the capacity for response to environment, even at 7½ weeks in man.

It might be pointed out that many activities of postnatal infants, which seem to be voluntary, are in fact reflex. Here, also, some are responses to the external environment (as to visual or auditory stimuli, for example), or to stimuli from within (as hunger, internal pain, etc.).

In the development of both fetal and neonatal activity, it is noteworthy that the two environments, external and internal, play an all-important role. Initially, the external environment appears to be the more significant, although even at fairly early ages internal environmental factors may affect the pattern of activity. There seems little doubt that, as the age of the fetus increases, the role of the internal environment assumes more importance.

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THE OXYGEN TENSION IN THE PLACENTAL BLOODS OF GOATS‡

The data presented here on the oxygen tension in the maternal and fetal bloods entering and leaving the placenta of the goat were obtained as a part of a wider investigation of the oxygen supply to the fetal kid. As the first step in the program we estimated the rate at which blood reaches the uterus of the goat at selected stages in gestation and the arterio-venous oxygen difference across that organ to obtain data on the oxygen consumption of the uterus and its contents.[13] Next we examined the characteristics of the oxygen dissociation curves of the fetal and maternal bloods of goats and the degree to which their respective affinities for oxygen were affected by changes in hydrogen ion concentration.[8] The results were related in two nomograms—one for fetal, the other for maternal blood—that permit the easy estimation of the oxygen tension in a blood sample when its percentage saturation with oxygen and its pH are known. These nomograms have been used to estimate the oxygen tensions in the bloods entering and leaving the maternal and fetal sides, respectively, of the placenta at different stages in gestation and in different circumstances. Finally, the results presented here served as the basis for the estimation of the transplacental oxygen gradient, i.e., the difference in the oxygen pressure in the maternal and fetal bloods entering and leaving the placenta.

MATERIALS AND METHODS

The goats used in this study were healthy, well-nourished individuals either Sanaans or Toggenburghs, whose breeding dates were known. They were starved for 48 hours before they were anesthetized with either intravenous sodium pentothal or chloralose in preparation for laparotomy.

Some of the animals were used for studies on uterine blood flow and metabolism before the blood samples used in this part of our work were drawn. In investigating the

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uterine blood flow it was our practice to draw—via indwelling plastic catheters—blood from a maternal artery and a uterine vein at a regular rate over a 20-minute test period just prior to the drawing of samples from the four placental vessels. These integrated samples drawn over the 20-minute period, when analyzed provided us with the average arterial and uterine venous oxygen contents respectively on the maternal side of the placenta and for the estimation of the maternal oxygen capacity. With these data, spot samples drawn from the same maternal vessels at the end of the test period could be compared. As these spot samples were drawn, others were drawn simultaneously or nearly so from an umbilical artery and an umbilical vein, in a loop of cord exposed by a small incision in the uterine wall. Each of these samples, of about 5 cc., was drawn under oil into a syringe whose walls had been moistened with a neutral heparin-fluoride solution to prevent clotting and glycolysis. The other blood samples, analyzed as a part of this study were obtained in the course of an investigation of the acid-base relations of the mother and fetus.[5] They were drawn simultaneously or nearly so from a uterine artery, a uterine vein, an umbilical vein, and an umbilical artery after the uterus had been delivered by a midline abdominal incision and opened by a small cut at right angles to the long axis of the horn.

The syringes into which the samples were drawn were capped and stored in ice water until analyzed. The pH of the whole blood was determined by means of a glass electrode and a Beckman meter standardized with a buffer of known hydrogen ion concentration. In some cases the pH was measured at 38° C.; in others it was measured at room temperature and corrected to 38° C. by means of Rosenthal's[18] equation:

$$\text{Blood } pH_{38} = pH_t - 0.0147\ (38 - t) \qquad (1)$$

where t is the temperature at which the pH is read. We determined by experiment that this correction is applicable to the bloods of adult and fetal goats. In some cases the pH of the blood was determined on only one member of the pair of fetal or maternal samples. The pH of the second was estimated from the measured value. If the sample measured was the more arterialized, the pH of the second was assumed to be 0.05 units lower; if it was the more venous, the pH of the arterialized blood was assumed to be 0.05 units higher. The error introduced by this practice would not appear to be significant so far as this study is concerned for we observed pH changes of this order occur in the other cases as the maternal and fetal bloods of goats as they pass through the placenta.[5]

The oxygen content of the blood was determined by Van Slyke's manometric method; the oxygen capacity was measured in the same way after exposure of a portion to air in a rotating syringe. These values were used to calculate the precentage saturation with oxygen. Finally, we estimated the oxygen tension in each sample by means of nomograms already published[6] that related oxygen tension to pH and percentage oxygen saturation for fetal and maternal goat bloods respectively.

RESULTS

Data were obtained on eleven goats (see Table 1) whose gestation ages ranged from 73-137 days (full term 145-50). Eight of the eleven, Nos. 1-5 and 9-11, were used in experiments on uterine blood flow before the fetal samples were drawn; Nos. 6-8 were sacrificed for this study and for the estimation of the transplacental carbon dioxide tension gradient. Of those

TABLE 1.

Number	Age Days	Vessel sampled	Oxygen capacity vols. %	Oxygen content vols. %	Coefficient oxygen utilization	Per cent saturation	pH	Oxygen tension mm. Hg
			A. "Normal" animals					
1	73	Ut. art.	12.2	12.2	23.0	100.0	7.42	> 90.0
		Ut. vein		9.4		77.0	7.39	47.0
		Umb. art.	10.0	1.5		16.5	7.35	10.8
		Umb. vein		9.1		90.6	7.40	> 45.0
2	116	Ut. art.	15.6	14.5	19.9	93.0	7.44	74.0
		Ut. vein		11.4		73.0	7.39	43.5
		Umb. art.	14.5	2.20		15.1	7.35	10.4
		Umb. vein		8.0		55.5	7.39	20.3
3	123	Ut. art.	13.1	13.0	26.0	99	7.44	> 90.0
		Ut. vein		9.6		73.2	(7.39)	43.2
		Umb. art.	15.7	3.1		19.1	(7.35)	11.6
		Umb. vein		9.6		73.2	7.40	27.0
4	125	Ut. art.	11.5	11.5	25.2	100.0	7.45	> 90.0
		Ut. vein		8.6		74.7	(7.40)	44.8
		Umb. art.	13.4	4.0		29.6	(7.35)	14.4
		Umb. vein		10.0		74.6	7.40	28.0
5	126	Ut. art.	11.3	11.1	20.4	98.2	7.45	> 90.0
		Ut. vein		8.3		73.4	(7.40)	43.0
		Umb. art.	11.9	5.1		42.8	7.32	18.5
		Umb. vein		7.7		64.7	(7.37)	24.3
6	133	Ut. art.	12.8	12.8	28.2	100	7.41	> 90.0
		Ut. vein		9.2		74.4	7.35	47.0
		Umb. art.	12.0	3.6		29.6	7.37	14.0
		Umb. vein		8.3		69.0	7.39	25.3
7	137	Ut. art.	10.1	10.0	19.8	99	7.44	> 90.0
		Ut. vein		8.0		80	7.32	54.0
		Umb. art.	9.9	2.4		24.1	7.33	13.2
		Umb. vein		6.2		62.1	7.38	23.0
8	137	Ut. art.	7.5	7.2	32.0	96.0	7.42	> 90.0
		Ut. vein		4.8		62.2	7.37	38.2
		Umb. art.	10.5	2.5		23.8	7.32	13.5
		Umb. vein		6.2		59.0	7.7	22.0
			B. Animals with low arterial oxygen saturation					
9	77	Ut. art.	15.7	13.1	28.0	83.3	7.30	60.5
		Ut. vein		8.7		55.3	(7.25)	39.0
		Umb. art.	9.0	3.7		18.8	(7.35)	11.5
		Umb. vein		7.2		30.0	7.40	13.5
10	95	Ut. art.	12.3	11.2	29.2	91.0	7.46	65.0
		Ut. vein		7.6		61.7	(7.41)	36.1
		Umb. art.	11.6	5.2		44.8	(7.35)	18.3
		Umb. vein		8.4		72.4	7.40	26.9
11	111	Ut. art.	12.7	11.6	43.3	91.4	7.35	75.0
		Ut. vein		6.1		48.0	7.30	33.0
		Umb. art.	11.5	3.9		33.9	(7.35)	15.4
		Umb. vein		6.5		56.5	7.40	20.5

used in the blood flow studies, Nos. 1-5 were considered to be in 'normal' condition as judged by their blood pressure, 90 mmHg or over, and arterial oxygenation, whereas Nos. 9-11 were judged to be in poor condition because of their low blood pressure and arterial saturation. The condition of those sacrificed primarily for this study, Nos. 6-8, was judged to be 'normal' on the basis of their reactivity and arterial oxygen level.

In the series of individuals designated as 'normal' the oxygen saturation of the arterial blood was 96 per cent or over, whereas that of the blood emerging from the uterus ranged between 62.2 to 80 per cent. The coefficient of oxygen utilization $\left(\frac{A\text{-}V}{A} \times 100\right)$ is correspondingly low. Only 19.8—32 per cent of the oxygen transported to the uterus was utilized by it and its contents. As a result of the low coefficient of oxygen utilization the oxygen pressure in the maternal placental capillaries was high. The oxygen tension in the uterine venous blood ranged from 38.2-54.0 mmHg; the arterial oxygen tension varied from 74 to above 90 (the accuracy of our estimation of oxygen tension in goat blood above 90 does not justify any more precise statement of the upper limit).

The oxygen saturation of the umbilical arterial bloods of fetuses delivered of the 'normal' does varied between 15.1 and 42.6 per cent; the range in the bloods from the umbilical vein was 55.5 and 90.6 per cent. These saturations corresponded in the umbilical artery to oxygen tensions between 10.4 and 18.5 mmHg and 20.3 to 45 in the umbilical vein.

In the three does whose condition was considered 'poor' the arterial oxygen saturation was 83.3-91.4; that of the uterine venous blood was 48.0-61.7. The lowest coefficient of oxygen utilization of the uterus was 28.0, the highest 43.3. The oxygen tension in the arterial blood was 60.5-75 mmHg. Turning now to the fetuses, the oxygen saturation of the blood of the umbilical vein ranged from 30.0-72.4 saturations that corresponded to oxygen tensions of 13.5-26.9. The saturations in the bloods of the umbilical artery were 18.8-44.8 corresponding to oxygen tensions 11.5-18.3.

DISCUSSION

As the data presented here were obtained on anesthetized does lying on their side, the reader will be concerned to know what relation the values obtained bear to those characteristic of normal unstressed individuals. Such was our own concern about this point that we delayed their publication until we could make some statement about it. We find that the arterial and uterine venous bloods of the does in our series designated 'normal' are saturated with oxygen to a degree similar to that which we find in unanes-

thetized individuals whose bloods were sampled via indwelling plastic catheters three to six days after the catheters were introduced. Though we have not determined as yet the degree of saturation of fetal blood entering and leaving the placenta in unanesthetized goats, there is no reason to assume that they would differ from those reported here inasmuch as the values on the maternal side of the placenta are similar in the anesthetized and unanesthetized individuals.

Our results indicate that in the 'normal' goat as in the unanesthetized sheep[10] the coefficient of oxygen utilization of the pregnant uterus continues to be low throughout the last third of the gestation period. The coefficient in the goats studied ranged between 19.8 and 32, values that correspond well with the limits of the variation observed in our sheep, i.e., 22.9-47.0. These results are in sharp contrast to those that have been obtained in similar studies on rabbits in which the coefficient of oxygen utilization tends to increase as term approaches and may reach values of 80.[4]

The circumstances that result in this difference in the utilization coefficient as observed in the sheep and goat on the one hand and the rabbit on the other have not been described, but some correlated information is of interest. The oxygen tension in the venous blood emerging from the uterus of the rabbit falls steadily as gestation advances after the 18th day.[3] In contrast, the oxygen pressure in the blood leaving the pregnant uterus of the 'normal' goat shows no such tendency; instead, it remains relatively high. In only one case was it below 43.0 mmHg, i.e., 38.2. Similarly, in our unanesthetized sheep, the oxygen pressure of the uterine venous blood tends to remain relatively constant, 40-45 mmHg, in the last third of gestation. And in unanesthetized ewes at altitude, this same level 40-43 mm was observed in the uterine venous blood despite the fact that at 15,000 feet where the data were obtained the arterial oxygen tension was about 47 mmHg for the barometric pressure fluctuated between 444.8 and 445.9.[11]

There is here the suggestion that in sheep and in goats the pregnant uterus during the last third of gestation is normally perfused at such a rate that the oxygen tension in the venous blood varies through relatively narrow limits. That being the case, the blood flow should increase as the oxygen consumption of its contents waxes with advancing gestation—and just such an increase has been observed.[12] In the rabbit, by contrast, the blood flow to the uterus reaches a maximum about the 22d day of gestation when the fetuses weigh circa five grams each.[3] The increased oxygen required by the rabbit fetus as development advances is met by increasing the coefficient of oxygen utilization; the result is a decline in the saturation and oxygen tension of the uterine venous blood. The implications and the consequences

of this difference in the coefficient of utilization are doubtless many but two appear fairly obvious: (i) The oxygen available to the uterus of the rabbit at the end of term is approaching a clear limit, whereas no such limit appears to be set for the pregnant uterus of the goat. (ii) Other circumstances remaining the same, less maternal heart work is required in the rabbit to deliver an equivalent volume of oxygen to its uterus than is necessary in the goat or sheep.

The pioneer work on the respiratory gas tensions in fetal blood was done by A. St. G. Huggett[7] in 1927 on goats near term, a study that demonstrated for the first time that the oxygen tension in the fetal blood was lower and the carbon dioxide tension higher than their respective levels in the maternal blood. The results presented above serve to confirm, though no confirmation was necessary, Huggett's conclusions and in the circumstances in which the techniques used were comparable our results are similar. In our study we were able to determine or estimate the pH of the blood samples and so to express the oxygen tension with a precision not possible at the time Huggett carried out his study. Accordingly, our results add some refinements but do not alter his fundamental observations.

As Huggett demonstrated, the oxygen tension in the fetal blood is normally much lower than that in the maternal; in our series the tension in the arterialized fetal blood was circa 45 to 70 mmHg lower than it was in the maternal artery. The difference in tension in the venous bloods was circa 20-40 mm. That is to say, the normal transplacental oxygen gradient in the goat is comparable to that observed in the sheep which has the same type of placenta.[8]

The factors responsible for this steep gradient have not been identified, but evidence is accumulating which indicates it is the result of mechanisms which can be altered quite rapidly and is not primarily determined by the structure of the placenta per se as had been suggested earlier might be the case.[8] Thus the gradient appears to be adjusted and maintained by the fetus to provide it with an adequate oxygen supply despite wide variations in the tension of that gas in the maternal blood reaching the uterus. The evidence on this point comes from three sources: (i) The data demonstrating that in ewes which live and reproduce at high altitudes, where the oxygen pressure in the arterial blood is circa 47 mmHg, the oxygen tension in the fetal blood and the oxygen consumption of the uterus and its contents in cc/kg/min. are comparable to sea level values.[11] (ii) Similarly, in sheep reared and kept at or near sea level until about the 100th day of gestation, the oxygen tension of the fetal blood is maintained at normal values when the mothers are exposed to decreased oxygen pressures in a low pressure chamber;[8] and

(iii) that offered by the goats in our small 'abnormal' series. Because of poor ventilation the arterial blood in these individuals was not well oxygenated. The arterial oxygen tensions were between 60.5 and 75 mmHg—normal is circa 95—and that of the uterine venous bloods between 33-39 as compared with 38.2-54.0 in the normal. Despite this decrease in the oxygen level in the blood perfusing the uterus, the oxygen tension in the fetal blood returning to the placenta in the three fetuses varied over the same range as that given for the fetuses of 'normal' does. Though the oxygen tension in the umbilical vein is lower than the lowest in the 'normal' series—13.5 mmHg as compared with 20.3 in the 'normal' series—the tension of that gas in the other two cases was within the lower limits of the 'normal' range. Further, though no direct measurement was made of the oxygen consumption of these fetuses, or those in the normal series for that matter, they appeared to be receiving oxygen at the same rate as those in the 'normal' series. Just prior to sampling the oxygen consumption of the uterus and its contents, as determined by measuring the arterio-venous difference across the uterus and the uterine blood flow, was in the range found to be characteristic of the cases in the 'normal' series studied in the same way, i.e., 8-10 cc.[13] Second, the total osmotic pressure of the fetal plasma did not differ from that of the maternal sufficient to suggest that the fetus was anoxic. Just prior to delivery, in an earlier study, we[9] observed that anoxia of the fetus was followed by a prompt and significant elevation in the total osmotic pressure of its plasma; the absence of such an increase we have taken to suggest that oxygen was reaching the fetus at a rate equal to its metabolic requirements.

Taken together, these three lines of evidence indicate that the transplacental oxygen gradient in the goat and sheep is subject to regulation, and that it is so adjusted that the oxygen supply to the fetus is maintained at the appropriate level despite changes in the oxygen supply to the uterus. That is to say, there appears to be a margin of safety in the functional capacity of the placenta with respect to the transfer of oxygen to the fetus.

SUMMARY

The oxygen tension in the maternal and fetal bloods entering and leaving the placenta have been estimated by relating the percentage oxygen saturation and the pH of the individual samples to previously published nomograms for these bloods in which oxygen tension, pH, and percentage saturation are related. The results demonstrated that in normal does during the last half of gestation the oxygen tension in the maternal arterial blood ranges from 74 to over 90 mmHg. In the uterine vein the oxygen tension for these same individuals varied from 38.2-54.0 mmHg. On the fetal side

the oxygen tension ranged between 20.3-45 mmHg in the umbilical vein and 10.4-18.5 in the umbilical artery. These fetal values are compared with those obtained from three cases in which the arterial blood of the doe was poorly oxygenated. The results are discussed.

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ORIGIN OF MYELOSCHISIS IN CHICK EMBRYOS INFECTED WITH INFLUENZA-A VIRUS‡

INTRODUCTION

It is well known that the central nervous system is involved in a major percentage of newborn and stillborn abnormalities in the human. In a study by Anderson,[a] defects of the central nervous system were found to occur in 49.5 per cent of congenital malformations incompatible with life. Frequent abnormalities are myeloschisis and anencephaly, along with the associated skeletal defects of spina bifida and cranioschisis, ranging in severity from a slight spina bifida in the lumbar region to an extensive craniorhachischisis.

The first major study of the embryonic development of human congenital malformations was done by Mall,[aa] who emphasized the fact that many of his early abnormal embryos had certain defects which he considered to be the forerunners of commonly seen malformations at birth. Since he found varying degrees of failure of closure of the neural folds in his youngest specimens, Mall concluded that the late fetal spina bifida type of monster is of similar origin, i.e., that abnormalities of newborn monsters originate early in embryonic development as deviations from normal development. Mall went one step further and attempted to discover the causative factor or factors in the origin of these early deviations by correlating wherever possible with clinical histories and placental conditions. From this study, Mall postulated that congenital malformations result from detrimental environmental influences such as nutritional impairment, and that this in turn is due to faulty implantation *in utero* caused by maternal disease.

More recently, considerable experimental work has been done with environmental teratogenic agents in the production of the spina bifida-

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myeloschisis and cranioschisis-anencephaly types of monster complexes. These abnormalities have been produced with x-rays by Kaven,[26] Russel,[39] and Auerbach[3] in the mouse embryo, and by Warkany and Schraffenberger[46] and Hicks[25] in the rat embryo; with anoxic conditions by Gallera,[18] Naujoks,[33] and Büchner[7] in the chick embryo, by Stockard[45] in fish eggs, and by Rothweiler[38] in Triton eggs; with various chemicals by Ancel,[1] Bellairs,[5] and Catizone and Gray[8] in chick embryos; with trypan blue by Gillman *et al.*,[24] Gunberg,[19] and Warkany *et al.*[47] in rat embryos, and by Hamburgh[23] in mouse embryos; with pantothenic acid deficiency by Lefevre-Boisselot[29] and Giroud *et al.*[15] in rat embryos; with excessive vitamin A by Cohlan,[9] and Giroud and Martinet[16] in rat embryos; with ultraviolet by Baldwin[4] in frog embryos; and with ultrasonic waves by Lutz and Lutz-Ostertag[31] in chick embryos.

While much of the recent work in this field has been concerned with such aspects as types of teratogenic agents, teratogenic dosage levels, critical times of action, and to some extent genetic influences, there have also been attempts to analyse the basic developmental mechanisms involved. Patten[34] found marked local overgrowth of neural epithelium in three cases of myeloschisis in human embryos and suggested that the open neural tube is the result of local overgrowth interfering with closure. Hamburgh[23] compared trypan-blue-induced neural abnormalities in mouse embryos with similar defects determined by genetic factors. He further demonstrated that trypan blue injected into pregnant mice does not penetrate the embryonic tissues but is arrested in the yolk-sac epithelium and in Reichert's membrane. Warkany *et al.*[47] demonstrated a relationship between myeloschisis and myelomeningocele in embryos of pregnant rats treated with trypan blue. When they arranged abnormal embryos and fetuses in order of increasing gestational age they demonstrated a progression of degenerative changes by which myeloschisis is transformed into myelomeningocele, thus strengthening the concept that an open neural plate is the basic anomaly of myelomeningocele in the human.

Abnormal development of the central nervous system in early chick embryos infected with influenza A has been reported by Hamburger and Habel,[20] Shear *et al.*,[40] Heath *et al.*,[24] and Williamson *et al.*[50] The neural defects consisted mainly of micrencephaly (collapsed encephalon) and abnormal axis twists. Kung[29] observed in microscopic studies of abnormal influenza-infected chick embryos, that development of brain tissue itself appeared normal except for a few small lesions and that the flattening of the encephalon, resembling a micrencephaly on gross examination, appeared to result from collapse of the brain wall associated with a lack of cerebral fluid.

During the course of a microscopic study of abnormal development in early chick embryos infected with influenza-A virus, it became evident that myeloschisis-type abnormalities develop in regions of the neural primordium which were in plate or fold stages at the time of inoculation (Robertson *et al.*[38]). The present study is concerned with an analysis of influenza-induced myeloschisis in an attempt to elucidate mechanisms involved in normal and abnormal neural tube formation.

TABLE 1. SURVIVAL OF EMBRYOS HARVESTED 24 HOURS AFTER INOCULATION WITH INFLUENZA-A VIRUS

Group	*Time of inoculation (in hours)*	*ID_{50} inoculated*	*Total embryos inoculated*	*Total embryos surviving*	*Per cent survival*
I	43-44	$10^{6.7}$-$10^{5.7}$	59	19	32.2
Controls*	43-44	.....	35	33	94.3
II	48-50	$10^{7.3}$-$10^{6.3}$	61	45	73.8
Controls*	48-50	.....	37	35	94.6
III	60	$10^{7.3}$	20	18	90.0
Controls*	60	.....	18	17	94.4

* Controls injected with normal allantoic fluid.

MATERIALS AND METHODS

A total of 230 chick embryos from flocks of New Hampshire reds was employed at incubation ages ranging from 43 to 60 hours. The PR8 strain of influenza-A virus was used, and the size of the inoculum was 0.05 ml. The inoculum was prepared from virus grown in the allantoic cavity of 12-day chick embryos for 48 hours. The infectivity titer was determined by inoculating 10-fold dilutions of virus suspension intra-allantoically into 12-day embryos and testing after 4 days for virus growth as indicated by agglutination of chicken erythrocytes by the allantoic fluids. The EID_{50} was calculated by the method of Kärber.[37] For inoculation into the early chick embryos the suspensions were diluted in buffered saline (pH 7.2) to the required infectivity titer.

The eggs were incubated in a forced-draft incubator at 98-99° F. The virus inocula were injected through the vitelline membrane and over the blastoderm as described previously for experiments with Newcastle disease virus by Williamson *et al.*[40] and Robertson *et al.*[38] Developmental stages were determined, according to Hamburger and Hamilton,[21] at the time of inoculation by observation of the embryos *in vivo* under 24x magnification. Such *in vivo* observations were not strictly accurate for the staging of any individual embryo but were adequate to estimate the average stages of various groups of embryos.

Approximately 24 hours after inoculation, the surviving embryos were harvested, fixed in Bouin's fluid, stained in dilute alum cochineal, dehydrated, and cleared. All embryos were then staged and examined under the dissecting microscope for abnor-

malities. Following this, the embryos were sectioned serially and stained with Delafield's hematoxylin and erythrosin for microscopic examination.

Of the 230 embryos used, 140 were inoculated with influenza A as described above. The inoculations were done at three different incubation ages as indicated in Table 1. The remaining 90 embryos were used as controls. They were divided into three control groups, and the embryos of each group were injected with normal 12-day allantoic fluid at the appropriate incubation ages corresponding to the three experimental groups. After incubation for the experimental period of 24 hours, all surviving embryos were

TABLE 2. MORPHOLOGICAL STAGES OF DEVELOPMENT OF EMBRYOS SURVIVING 24 HOURS AFTER INOCULATION WITH INFLUENZA-A VIRUS BASED ON STAGING OUTLINED BY HAMBURGER AND HAMILTON[21]

Group	*Time of inoculation (In Hours)*	*Stages at time of inoculation**		*Stages 24 hours after inoculation*	
		Range	*Mean*	*Range*	*Mean*
I	43-44	8-13	11.4	14-17	15.9
Controls†	43-44	9-13	11.4	14-17	16.0
II	48-50	10-14	12.4	13-18	16.7
Controls†	48-50	10-14	11.9	14-18	16.9
III	60	12-16	14.2	17-21	19.4
Controls†	60	12-16	14.3	17-21	19.4

* Incubation temperature was 98-99° F.
† Controls injected with normal allantoic fluid.

harvested, fixed, stained and cleared for staging, and examined for grossly visible abnormalities under the dissecting microscope. Representative embryos were sectioned serially for microscopic examination.

EXPERIMENTAL

Survival

The data in Table 1 show the number of embryos surviving in the experimental and control groups 24 hours after inoculation. It is noteworthy that the survival rate is high (94.3-94.6 per cent) in all three control groups. In the virus-inoculated groups, however, survival rate is only 32.2 per cent in the youngest group, 73.8 per cent in group II, but 90 per cent in the oldest group. Within the variations inherent in the experimental approach (differences in flocks from which eggs were obtained, seasonal variations, etc.) the decrease in lethal effects of influenza A appears to be correlated with increasing age of the embryos within the time range of our experiments. This interpretation is strengthened by the fact that the virus titer employed was somewhat higher the older the group (see Table 1) and pre-

vious work (Williamson *et al.*[20]) has shown a greater effect on survival rates with higher titers.

Morphological stages of development

Embryos were staged according to the staging criteria of Hamburger and Hamilton.[21] Since different laboratories use different types of incubators and slightly different incubation temperatures, any discussion of developmental processes should be based on morphological stages instead of incubation times. In Table 2 the range in stages in each experimental and control group is given, and in each case the mean stage has been calculated in the usual manner. It should be emphasized that these means are not accurate

TABLE 3. NUMBER AND PERCENTAGE OF SECTIONED INFLUENZA-INOCULATED EMBRYOS WITH MYELOSCHISIS

Group	*Time of inoculation (in hours)*	*Number of embryos*	*Number of embryos with myeloschisis*	*Per cent of embryos with myeloschisis*
I	43-44	17	8	47
II	48-50	30	10	33
III	60	16	1	6

arithmetic means since there is no basis for assuming that successive stages are arithmetically equidistant. However, the means do give a rough estimate of the morphological stage of development of each group as a whole and thus simplify the present discussion.

The data in Table 2 indicate a similar range in stages in both experimental and control embryos in each of the three groups, both at the beginning of the experiments and at the termination. This means, insofar as the criteria for staging used by Hamburger and Hamilton[21] are concerned, that the surviving influenza-inoculated embryos reached the same stage of gross morphological differentiation as the corresponding control embryos. However, observations indicate a noticeable stunting of growth in the virus-inoculated embryos as compared to control embryos.

Abnormalities in surviving embryos

In a previous publication by Williamson *et al.*,[20] it was noted that a high percentage of embryos, inoculated with a critical inoculum of influenza A at 48 hours' incubation, showed grossly discernible abnormalities 24 hours later. The defects consisted of absence or retardation of the lens and otocyst as well as abnormalities involving the neural tube such as twisted axis and

collapsed encephalon. A similar high incidence of abnormalities involving the same developing organs is evident also in the groups of embryos employed in the present study. In addition, however, when the 63 serially sectioned embryos were studied, another defect of special interest was observed with a much higher incidence in younger than in older embryos.

TABLE 4. TABULATION OF HAMBURGER-HAMILTON AND SOMITE STAGES WITH CRITICAL EVENTS[1] IN THE FORMATION OF THE NEURAL TUBE CORRELATED WITH THE THREE GROUPS STUDIED

HAMBURGER-HAMILTON STAGE	NUMBER OF SOMITES	DEVELOPMENTAL POSITION OF GROUP AT BEGINNING OF EXPERIMENT[2]	DEVELOPMENTAL POSITION OF GROUP AT TERMINATION OF EXPERIMENT[2]	CRITICAL EVENTS IN NEURAL TUBE FORMATION
7	1			neural folds visible in head region
8	4			neural folds meet at level of midbrain
9	7			neural folds fuse in midbrain region
10	10			fusion extends cephalad and caudad
11	13	I		anterior neuropore closing
12	16	II		posterior neuropore
13	19			present and closing[3]
14	22	III		
15	24-27			
16	26-28		I	caudal neural tube forming from end bud (tail bud)[4]
17	29-32		II	
18	30-36			
19	37-40		III	
20	40-43			
21	43-44			

[1] According to Hamburger and Hamilton,[21] Hamilton,[28] Romanoff,[27] and our observations.
[2] Group designation is placed opposite mean stage, with vertical line indicating range.
[3] From observations in control series it is evident that the so-called posterior neuropore comes into existence as such at stage 11 and is closing during stages 12 and 13, but often persists as a minute opening which may be present into stage 16.
[4] According to Gaertner.[13]

This type of abnormality will be referred to as myeloschisis and involves regions of the neural primordium where failure of neural tube formation appears to be the basic defect.

Incidence of myeloschisis

The number and percentage of influenza-inoculated embryos in which myeloschisis was noted are tabulated in Table 3. In group I, inoculated at

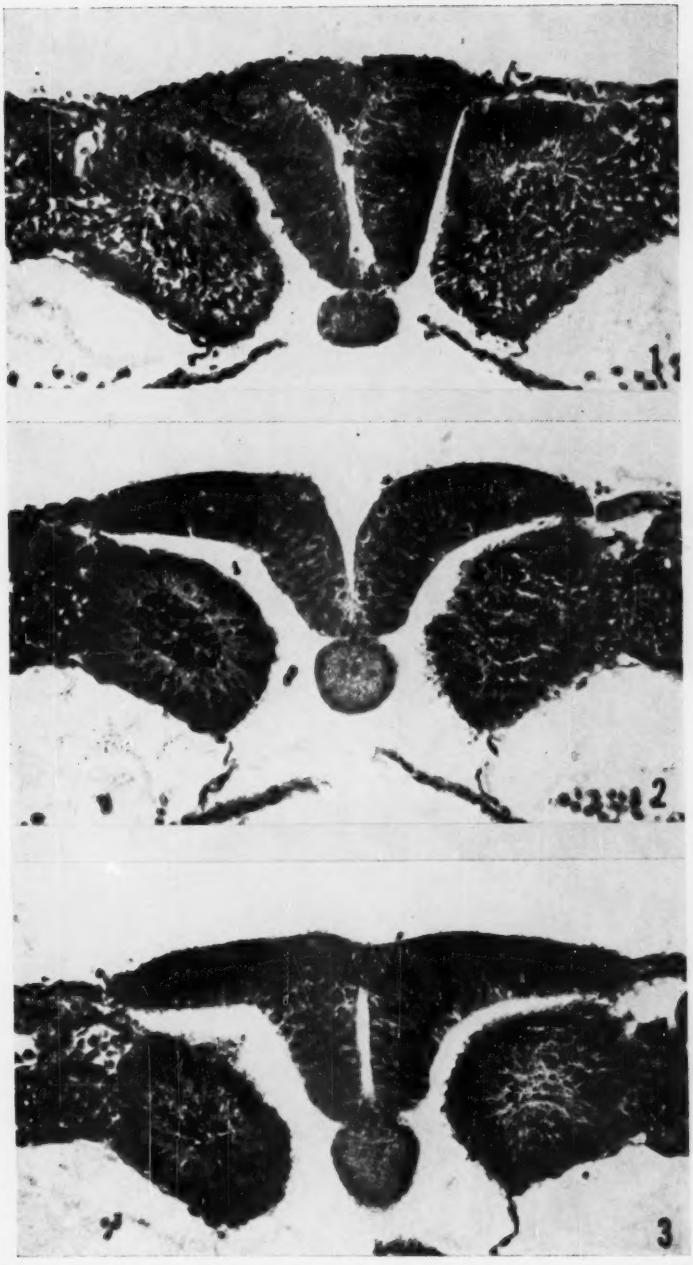

FIG. 1. Cross-section of abnormal neural primordium at level of somite 13 in influenza-infected embryo of group II. The embryo was inoculated at 48 hours' incubation (temperature 98-99° F.) and fixed at 60 hours (H. and H. stage 13, 19 somites). x240.

FIG. 2. Cross-section of same embryo as in Fig. 1, but further caudal, at level of somite 16. x240.

FIG. 3. Cross-section of same embryo as in Figs. 1 and 2, but further caudal, at level of somite 17. x240.

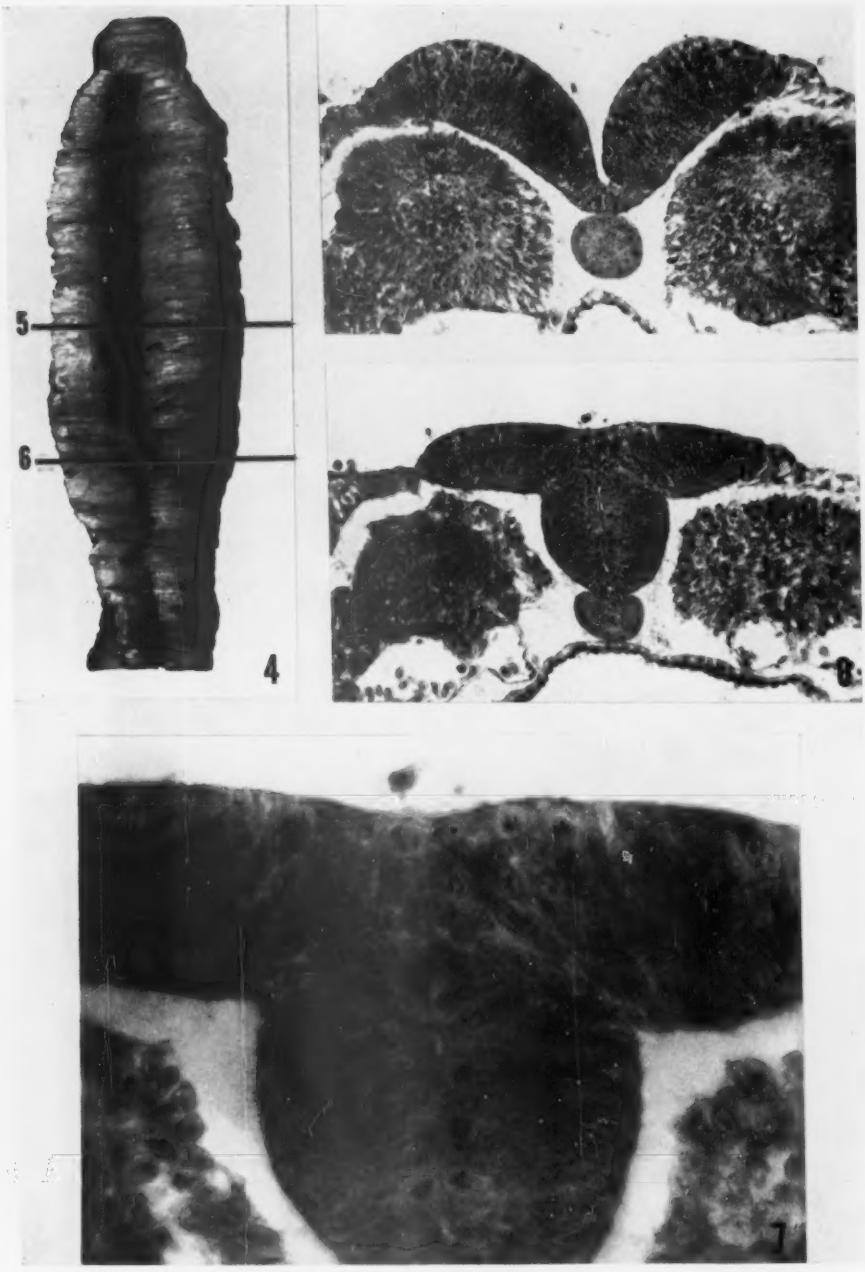

FIG. 4. Wax reconstruction model of abnormal portion of neural primordium in influenza-infected embryo of group II. The embryo was inoculated at 48 hours' incubation (98-99° F.) and fixed at 60 hours. (H. and H. stage 16, 27 somites.) Cross lines indicate levels of Figs. 5 and 6. x80.

FIG. 5. Cross-section of same embryo as in Fig. 4, at level of somite 24. x200.

FIG. 6. Cross-section of same embryo as in Fig. 5, but further caudal, at level of somite 27. x200.

FIG. 7. Same as Fig. 5, but at higher magnification. x550.

43-44 hours' incubation, 8 of 17 embryos (47 per cent) show myeloschisis. In group II, inoculated only a few hours later (48-50 hours), 10 of 30 embryos (33 per cent) are involved. In group III, which is the oldest group inoculated (at 60 hours), only one of 16 embryos (6 per cent) has the defect. Thus, it is evident that the incidence of myeloschisis in influenza-inoculated embryos is higher in younger than in older embryos, at least in the age range of 43 to 60 hours. In addition, the cephalocaudal extent of the defect generally is more extensive the younger the embryos.

Cephalocaudal extent of myeloschisis

The correlation of progressive restriction in extent of involvement of the neural tube with increasing age at inoculation closely parallels events in the development of the neural tube (see Table 4). The closure of the neural folds to form the neural tube normally begins during stage 8 at the level of the midbrain and progresses thence both cephalad and caudad. The formation of the cranial portion of the neural tube is complete when the anterior neuropore closes off, and this is accomplished by stage 12. Failure of complete closure of the encephalon was observed in some of the virus-inoculated embryos of groups I and II in addition to the collapsed micrencephalic condition. Further investigations on the abnormalities of the developing brain will be reported later.

In the future spinal cord region of the neural primordium, the neural folds normally meet and fuse in a progressively cephalocaudal direction with complete closure of the caudal end of the neural tube by stage 17. It should be noted, however, that all of the neural tube is closed off except for the posterior neuropore during stage 11. The posterior neuropore closes off during stages 12 and 13 according to gross observations, but microscopic examination indicates that a minute opening may sometimes persist as late as stage 16. The correlation of these normal developmental events with the time of virus inoculation and subsequent disturbance of formative processes indicates that (i) myeloschisis occurs only in embryos inoculated prior to the stage at which the neural tube has formed in its entirety, and (ii) the defect is present only in those regions not closed off into a tube at the time of inoculation.

The abnormal neural primordia vary in shape from an almost flat plate to a neural-fold type of configuration, or curve in a direction opposite to normal; or the presumptive basal-plate regions are rolled up into a tube with the presumptive alar-plate regions remaining flattened and exposed dorsally. There is, however, a consistent cephalocaudal pattern of shape variation regardless of the length of the defect. At the cephalic end of the defect the neural primordium is U-to V- shaped for a short distance (Fig.

1). Caudal to this the V-shape is broader (Fig. 2) with the deep surface of the neural epithelium more extensive than the free surface. This portion is variable in length depending on the length of the entire defect, but is longest in those embryos with more than the minimum defect, and the broadening of the V shape is more accentuated caudally. This broad V shape may extend to the caudal end, but in many the caudalmost region is T-shaped with a lumen in the stem of the T. This represents a closure of the presumptive basal-plate regions, with the presumptive alar-plate regions remaining flattened (Fig. 3). Figure 4 is a photograph of the dorsal surface of a model of the caudal region of the neural primordium, extending caudally from the level of somite 17, in an influenza-inoculated embryo from group II. The two indicator lines show the levels of the sections in Figures 5 and 6.

Histopathology

The abnormal neural epithelium is characterized by varying degrees of (i) cellular degeneration, (ii) sloughing of degenerated material, and (iii) piling-up of nuclei in the germinal zone. These manifestations of viral activity are more extensive in the presumptive alar-plate than basal-plate regions.

The cellular degeneration includes cytoplasmic degeneration but is characterized chiefly by an increase in nuclear and/or other basophilic material in the form of irregular pycnotic masses (see Figs. 1-3, 5-7). In some regions there is extensive sloughing of this material, especially at the cephalic end of the defective area where the neural primordium is U- or narrow V-shaped (Fig. 1). In other regions sloughed material is not extensive or may be absent. In all cases the basal regions are less involved than the more dorsal regions which represent the apical regions of the neural folds.

The piling-up or accumulation of a greater than normal number of nuclei in the germinal zone of the neural epithelium is marked in the dorsal (alar) regions as compared to the ventral (basal) regions. This difference is most marked in the caudal T-shaped primordium in which the basal region has closed off to form a tube (Fig. 7). The possible significance of this difference will be discussed below.

Growth

As stated previously, the virus-inoculated embryos develop to approximately the same over-all stage of morphological differentiation but not to the same size as control embryos. For example, the same number of somites develop as in the corresponding control embryos, but each somite

is somewhat shorter, and the difference in length of the neural primordium is roughly proportional. In an attempt to discover if localized changes in growth pattern in the neural primordium might be correlated with the development of myeloschisis, it was necessary to compare cross-sectional areas of the experimental with the control. This was done by making serial projection drawings of the neural primordium extending from the

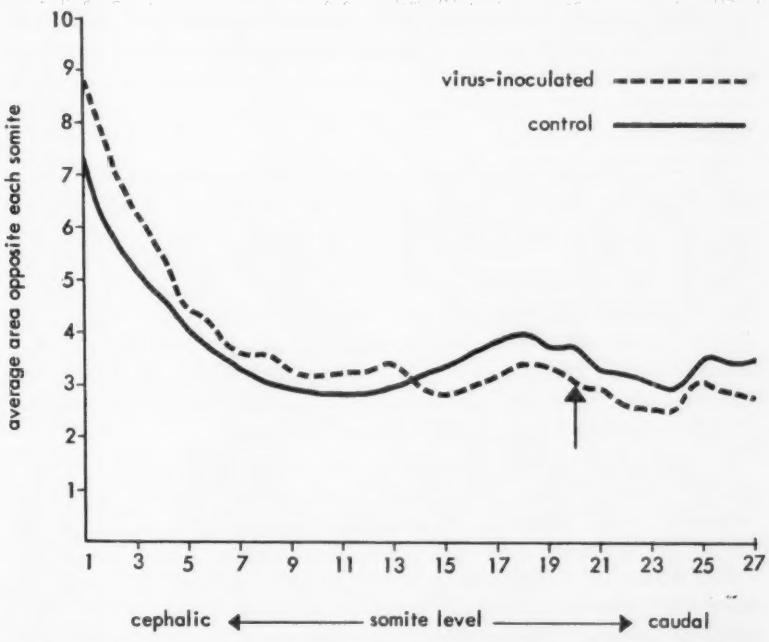

FIG. 8. Curves indicating average cross-sectional areas in the neural segments opposite the somites. The areas are expressed in square inches for the projection drawings done at x300 magnification. The arrow indicates the average anterior extent of myeloschisis in the three virus-inoculated embryos measured. Curves for individual embryos vary only slightly from the average.

level of the cephalic end of the first somite to the caudal end of the last somite. The areas were then determined from planimetric measurements and averaged for the segments of the neural primordium opposite each somite. These measurements were made on three virus-inoculated and three corresponding control embryos from group II, and the averaged results are depicted by graph in Figure 8. It is evident that the average cross-sectional area is greater in the experimental than in the control in the

portion of the neural tube opposite the first 13 somites. However, the caudal portion extending from the level of the 14th somite is less extensive in average cross-sectional area in the experimental, and the myeloschisis is in this area extending caudally from the 20th somite. It is obvious, then, in these embryos that the region of myeloschisis is not one in which overgrowth is a phenomenon, at least at the stage at which these embryos were harvested. This conclusion is strengthened by the fact that a comparison of normal and abnormal regions in the experimental indicates that the average cross-sectional area in the abnormal region is less than in most of the neural tube anterior to the defect.

DISCUSSION

The basic mechanisms involved in the process of neurulation (neural tube formation) are not clearly understood, but there is a considerable amount of experimental work which can be related to the problem, either directly or indirectly. A basic consideration is the fact that during the process of folding and closure of the neural epithelium the deep (proximal) surface becomes progressively more extensive than the free (luminal or distal) surface. This difference was originally thought to be due to a differential in fluid uptake by the neural epithelium (Glaser,[17] Hobson[28]), but density studies by Brown *et al.*[6] on amphibian embryos indicate that folding processes can occur without differential fluid imbibition.

A differential in proliferative activity was thought by Derrick[11] to play a role in neurulation in the chick embryo since she found a greater number of mitoses in lateral regions of the medullary plate just prior to and during neural fold formation. However, Corliss and Robertson[10] found no lateral-medial differential in the medullary plate just prior to neural fold formation but a relative increase in mitotic density in presumptive basal-plate as compared to alar-plate regions during neural fold and tube formation. The mitotic density remains higher in the ventral half than in the dorsal half of the formed neural tube at least until 72 hours according to Hamburger.[19] From these observations, it seems apparent that increased mitotic density cannot be correlated directly with the process of neural fold formation. This conclusion is strengthened by the fact that the alar regions are more susceptible to influenza-A virus at a developmental time when the mitotic density is less than in the future basal regions. This seems to indicate that the differential susceptibility is due not to a mitotic difference but to some other mechanism which is essential for neurulation, and that this mechanism, whatever it may be, operates more actively in the alar than in the basal regions.

If neurulation results from some mechanism operating more actively in future alar tissue, it is noteworthy that nuclei accumulate in the germinal zone of presumptive alar tissue in areas of myeloschisis in influenza-infected embryos. This abnormal piling-up of nuclei may be the result of disruption of a mechanism normally concerned directly or indirectly with neural tube formation. Sauer[40] indicated from histological studies of the early neural tube that the nucleus of the neural epithelial cell migrates towards the germinal zone as it rounds up in prophase. Prior to the beginning of mitosis the nucleus is ovoid in a long slender cell which is anchored firmly at the luminal surface by a terminal bar network. The rounding-up of the nucleus at the beginning of prophase results in the rounding-up and consequent shortening of the cell. Since anchorage is firm at the luminal surface the shortened cell comes to lie in the germinal zone where the mitotic division is completed. Following this, the daughter nuclei migrate towards the deep surface of the epithelium and become ovoid nuclei in tall, slender pyramidal cells which are narrow at their apical ends and broader towards their deep ends. This concept of nuclear migration has been strongly confirmed recently in separate studies using three different technical procedures: the colchicine method by Watterson *et al.*,[42] microspectrophotometric measurements for DNA by Sauer and Chittenden,[41] and radioautographic demonstration of DNA after treatment with tritiated thymidine by Sauer and Walker.[43] While these studies have been done at stages of development shortly after neural tube formation, it seems likely that the same mechanism is operating prior to and during neural tube formation when all of the neural epithelial cells are considered to have proliferative potencies. The differential in susceptibility to influenza-A virus at the time of neural fold activity may be the result of a more rapid rate of nuclear migration in the alar regions. The lower mitotic density in the germinal zone of the alar wings at this stage (Corliss and Robertson[30]) could also be considered as the result of a more rapid rate of nuclear migration before and after cell division. It is interesting to note that Watterson *et al.*[42] found almost every cell in the alar region to be in metaphase when a colchicine block was maintained for 7½ hours in 2-day chick embryos.

While it is possible that nuclear migration may result in changes in cell shape which in turn result in the folding process of neurulation, it is also possible that all of these phenomena may result from differential contraction of intracellular and/or intercellular fibrous elements. In the neural tube of early chick embryos, Spratt[44] has demonstrated the presence of specifically oriented intercellular fibers which are cytoplasmic or plasma membrane extensions of the neural epithelial cells. Also, it is possible that the terminal

bar network described by Sauer[40] may play a role in neurulation by having a restrictive effect on the ends of the epithelial cells at the luminal surface.

The characteristic myeloschisis in the influenza-infected chick embryos appears to be due to an inhibition of formative processes, involving perhaps one or more of the mechanisms discussed above. However, the phenomenon of overgrowth should be considered since Patten[34] postulated this as the probable mechanism involved in the origin of similar abnormalities in the human embryo. Hamburgh[22] found hypertrophy of neural tissue in regions of neurulation failure in embryos from pregnant mice subjected to trypan blue treatment. Warkany *et al.*[47] have described the typical eversion of the open tube induced by trypan blue in the rat and have demonstrated clearly the extensive overgrowth of neural tissue. The cross-sectional view of the open primordium in the influenza-inoculated chick embryos is of a similar nature, except that the appearance of hypertrophy does not seem marked, at least from visual microscopic observations. The measurements described above indicate that the abnormal regions in these virus-inoculated embryos are not hypertrophied, but are in fact somewhat smaller than comparable areas in control embryos. It should be emphasized that all of these embryos were fixed for microscopic study 24 hours after virus inoculation, and there is the possibility that hypertrophy might occur in the open neural epithelium of embryos infected over longer periods of time. However, it is evident that the origin of the typical myeloschisis in influenza-infected chick embryos is due not to hypertrophy of neural epithelium but to an inhibition of the mechanism(s) responsible for the process of neural tube formation.

SUMMARY

Chick embryos at incubation ages ranging from 43 to 60 hours were inoculated over the blastoderm with the PR8 strain of influenza-A virus. Survival rates at 24 hours after inoculation indicate that the younger embryos are more susceptible to the lethal effects of the virus than the older embryos. Those surviving, however, develop to the same stage of gross morphological differentiation as control embryos, but with some growth inhibition.

Abnormalities occurring in the embryos 24 hours after virus inoculation include collapsed encephalon and myeloschisis as well as absence or retardation of lens and otocyst. The myeloschisis was studied intensively in serially sectioned embryos.

The incidence as well as the cephalocaudal extent of myeloschisis is higher in the younger than in the older embryos. It is evident that myeloschisis occurs only in embryos inoculated prior to the stage at which the neural

tube has formed in its entirety, and further, that it is present only in those regions of the neural primordium not closed off into a tube at the time of inoculation.

The abnormal neural epithelium is characterized by varying degrees of (i) cellular degeneration, (ii) sloughing of degenerated material and (iii) piling-up of nuclei in the germinal zone. These manifestations of viral activity are more extensive in the presumptive alar-plate than basal-plate regions.

Possible mechanisms in normal neural tube formation are discussed in relation to the virus-induced myeloschisis. Such mechanisms include nuclear migration, changes in cell shape, as well as differentials in proliferative activities and contractility of intracellular and intercellular fibrous elements. It is thought that the virus-induced defect results from an inhibition of one or more of these mechanisms. Measurements of serial projection drawings of cross-sectional views of the neural primordium indicate that overgrowth is not the mechanism involved in the origin of the typical influenza-induced myeloschisis.

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REGENERATION OF THE LENS, IRIS, AND NEURAL RETINA IN A VERTEBRATE EYE**

In all classes of vertebrates the lens arises in the embryo from the surface ectoderm, which normally comes into contact with the early optic vesicle as it grows out from the wall of the brain on either side. Spemann[8] in his Silliman Lectures reviewed his experiments and those of others on the embryonic eyes of amphibians which showed that for the lens this contact has particular significance. If the contact fails to take place, no lens develops. If the normal presumptive lens-forming ectoderm is replaced by ectoderm taken from other regions of the body of the same or even another species of embryo, it will also be induced by the underlying eye tissue to form a lens rather than the skin it would have formed had it remained on the donor.

A study of experimentally produced cyclopian monsters in amphibians[3, 4, 38] also showed very well the influence of the early developing eye upon the origin of lens tissue. The degree of fusion of the eyes determines the degree of fusion of the induced lenses. When the two eyes are completely fused into a large median-placed one, a single correspondingly large lens is induced if contact is made with the surface ectoderm. If there is no contact, a lens fails to develop.[38]

When a lens is fully differentiated, it is suspended by zonular fibers extending from the ciliary processes. But if the original lens is detached from these fibers and entirely removed from the eye, leaving no remnant of lens tissue, what are the chances that it would be replaced by some form of regeneration? It would appear at first that replacement of the lost lens is not possible.

Twenty years ago[30] we found that fragments of the lenses of various amphibians and fishes, implanted into the eye, have the ability to organize and form new lens fibers if they included a normally high proliferating region of the epithelium along the equator of the lens. The organization and growth were best in the eyes of younger animals. In some adult salamanders

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which possessed the ability to regenerate lens from iris tissue, lens fragments did not survive. We shall describe these experiments later.

In 1900 Randolph reviewed the work of earlier investigators who claimed regeneration of the crystalline body of the rabbit lens after its removal. The evidence was chiefly based upon macroscopic observations. They reported that regeneration did not appear unless the lens capsule was present. Later, when microscopic methods became available, the cells at the equator of the lens were suggested as the source for the replacement of the host lens tissue. However, not much careful work had been done up to the time of Randolph's experiments. He removed the lenses from 20 rabbit eyes and also found that when the entire lens was removed it was not replaced. When the capsule and a portion of the crystalline body remained behind there were in some cases evidence of lens regeneration. The volume of the regenerated lenses was found equal to the original lens in one case 11 months after operation. He concluded that the formation of new lens tissue was brought about by both the hypertrophy of lens fibers left after operation and by the continued development of the lens-forming cells in the nuclear zone along the equator of the lens.

Recently Chanturishvili[2] and his coworkers, Russian investigators, have introduced small fragments of cytolyzed fetal tissues into the emptied lens capsule of adult rabbits and mice. It is claimed that normal regeneration of the crystalline body takes place after a long time only in the presence of the products of the cytolyzed tissue. In fact they believe that the entrance of cytolyzed foreign cell material is important for the development of the embryonic lens in the beginning.

Two British investigators, Stewart and Épinasse,[12] have repeated the Russians' experiments on a few rabbits. Their gross observations by slit lamp bio-microscopy up to 11 months after operation have led them to believe that their findings may confirm those of the Russian investigators on rabbits. They kindly brought their rabbits from the University of Hull to Cambridge University, where I was visiting in August 1959, and demonstrated the conditions seen in the living eyes. It will now be most interesting to see what the gross dissection of these eyes and the microscopic examinations reveal. Their experiments are being continued with this in mind.

Among our earlier experiments dealing with regeneration in lens fragments[38] and lens removal in various species of amphibians, there were many eyes which possessed no ability to replace a lost lens by any form of regeneration. However, one adult newt of this group which did replace a lost lens, the common aquatic-dwelling, vermillion-spotted salamander, *Triturus v. viridescens,* belongs to a limited group of salamanders in which a lens will regenerate by a budding process from the dorsal pupillary margin of

the iris after its original lens is completely removed. This phenomenon was first discovered by Colucci in 1891 and has been studied by various investigators. Their findings have been recently reviewed by Reyer.[9] A brief summary will therefore be made of the present author's extensive study of regeneration of the eyes of the adult vermillion newt mentioned above.

When the original lens is removed through a slit in the cornea of the eye of the adult newt,[88] (Figs. 3-22), the pigment epithelial cells along the dorsal pupillary margin of the iris at 12 o'clock gradually thicken, lose their pigment, and proliferate, by a budding process, an epithelial vesicle which gradually differentiates into a lens. In about a month it is detached from its source of origin, remains suspended behind the pupillary space, and after many months of growth it attains the size of the original lens which was excised.

Lens regeneration is inhibited by the presence of living lens tissue even though the lens tissue is transplanted from another species.[90] Other tissues do not. A mechanical factor exerted by a spherical body, such as a wax or glass bead, plays no inhibiting role. If the normal lens is left in place, and the dorsal iris is isolated from it by inserting a thin sheet of plastic membrane to prevent the dorsal iris from being bathed by aqueous humor in contact with the intact lens, lens regeneration is then released. Aqueous humor obtained from an eye containing a normal lens and injected daily into a lentectomized eye prevents lens regeneration only as long as it is injected.[88] These and other experiments demonstrate that the living lens tissue gives off some substance into the aqueous humor that inhibits lens regeneration.

We have also found that the presence of the neural retina is essential for the release of lens regeneration as soon as the inhibiting factors are eliminated. For as long as two months[89] dorsal iris tissue was isolated from the neural retina by plastic membranes inserted into lentectomized eyes, by permanent removal of the retina,[85] and by keeping iris membranes in tissue culture for some time.[80] Lenses regenerated as soon as the isolated iris membranes were transplanted to lentectomized eyes in the presence of a normal retina. Some experiments indicated that the amount of lens tissue regenerated depended upon the amount of neural retina present.

Some of our experiments showed that the neural retina influences the polarization of a regenerating lens. This was revealed[81] by reversing the dorsal iris and by controlling the position of a patch of retina tissue with respect to the iris. The lens-forming pole always pointed towards the area occupied by the neural retina membrane, just as it does during normal development.

By transplanting various segments of the iris along the pupillary margin to lentectomized eyes it was found that the lens-forming cells are located only along that part of the pupillary margin in the dorsal iris tissue.[39] By producing accessory openings in all regions of the iris[40] we found that small lenses developed from the margins of the artificial openings (Fig. 1) which were located over a broad area of the dorsal iris region, but in the dorsal iris only. We also found[34] that when permanent openings were made in the dorsal retinal wall by implanting pieces of plastic membranes, small lenses often developed from the retinal pigment cells from the border of these openings. Transplants of retinal pigment membranes placed in lentectomized eyes also will give rise to small lenses, only if the grafts came from the dorsal region.[7, 35] Therefore it is quite clear that during the development of this newt eye, presumptive lens-forming cells become localized over a wide area in the dorsal region of the eye.

In many groups of vertebrates very little is known about the regenerative capacity of the iris. In the few fishes which we have examined so far there has been no evidence of iris regeneration. We have experiments in progress for studying the possibility of regeneration in the iris of birds and some of the smaller mammals. These studies are of particular interest for there is yet no good explanation why the epithelial and connective tissue elements of the human iris exhibit no capacity for regeneration.

In the amphibians, especially in the salamanders, the situation is quite different. Varying amounts of iris tissue were removed in the adult newt eye.[31] The wound margin of the iris usually heals readily and becomes thicker within a few days. Then the inner and outer epithelial layers of cells begin proliferating a thin, sparsely pigmented membrane. This advances quite rapidly but the length of time required for complete regeneration depends largely on the amount of iris excised. The connective tissue stroma on the outer surface of the iris also keeps pace with the advancing epithelial membrane. When the whole dorsal half of the iris was removed up to the margin of the ora serrata,[31] regeneration was complete in about six weeks. In all of these cases a new lens regenerated from the regenerating dorsal iris at any time the normal lens was removed.

In another group of experiments the entire iris was excised along with adjacent rim of the retina at the ora serrata.[28] This left no remnant of iris tissue. Most of the eyes slowly recovered, although for a long time they were much smaller than normal. The retinal pigment cells bordering the wound slowly proliferated to form a new iris membrane. Some eyes at the end of three months (Fig. 2) showed a completely restored iris. In these cases also the regenerating dorsal iris regenerated a lens from the regenerating dorsal iris whenever the original lens was removed.

Regeneration of the optic nerve in any vertebrate eye would in the first place depend upon the status of the retina and in particular on the ganglion cells which supply the nerve fibers of the optic nerve and which connect with the proper stations in the optic tectum. The effects of a temporary loss of blood supply to the functional retina are usually severe. The entire retina may degenerate rapidly, and if there are no cells capable of regenerating a new retina, permanent blindness would result as in fishes.[14]

In order to study the development of the visual mechanism, in 1924, I explored among the vertebrates for a functional eye which could be successfully transplanted with return of vision. The eyes of larval and adult salamanders provided a tool for these studies. For earlier stages in the development of the mechanism, operations were also performed on embryos of the salamander. The author's results have been recorded in many publications and in several motion picture films. References to them can be found in more recent publications,[15-19, 23, 24] and those cited in this discussion.

It was found in transplanted eyes of larval salamanders, including those exchanged between animals of different species, that the retina survived and the optic nerve fibers grew rapidly back to the brain again, to bring about return of vision. However, in transplanted eyes of adult newts, the neural retina completely degenerated quite rapidly, although the blood circulation was re-established early after operation. The retina pigment epithelium survived (Fig. 4). These cells changed from flat to columnar in shape, and by mitosis an inner single layer of nonpigmented cells was formed. These cells by rapid proliferation gave rise to a thick membrane which differentiated into a new neural retina (compare Figs. 3 and 5). Nerve fibers from the new ganglion cells made their exit from the nerve head and followed the course of the old degenerated optic nerve to reach the optic centers of the brain. Usually return of vision can be demonstrated between two and one half to three months after this type of operation where the neural retina is eliminated by degeneration.

Regeneration is much more rapid if the entire neural retina is removed through a dorsal slit in the eye wall.[16] The denuded retinal pigment cells regenerate a new neural retina in the same way, but more rapidly than in the grafted eye. When retinal pigment cell membranes are implanted but isolated in the chamber of other eyes, they also form neural retina tissue.[18, 25] even though they are not vascularized.

The capacity for regeneration of the retinal pigment cells in the eyes of the newts is very great. It was found that the same eye[20] can be transplanted as many as four times (Fig. 8) to different hosts with regeneration of neural retina and return of vision each time. It was also shown that the eyes of the adult newt can be successfully transplanted after seven days of refrigera-

tion.[15a] The newly regenerated retina appears to be permanent, for some transplanted eyes have remained fully functional and possess normal appearing retinae for as long as four years and seven months after operation (Fig. 6). Although the transplanted eyes may be somewhat smaller than they were originally their appearance is quite similar to the normal eye (Fig. 7).

We have grafted the eyes of many different species of adult salamanders to the common vermillion spotted newt, where they function as a visual organ on the new host for a long period of time. For this to be accomplished, it means that the organization in the eye and the brain in each case must be quite similar.

Such experiments as these made it possible to exchange eyes between species which normally exhibit marked differences in visual acuity. When an eye from an animal with a normal low visual acuity is transplanted to a newt which normally possesses a higher visual acuity, the new host with that eye demonstrates a sharper vision than the donor, but not as high as with its own eye. The brain in this instance seems to have contributed something to the quality of vision. Studies of this nature have also been made by the author in eyes exchanged between larvae of different species, where the retinae do not degenerate. The optic nerve in these cases rapidly regenerates and then establishes vision. However, to restrict regeneration only to the optic nerve in the eyes of adult newts it is essential to cut the optic nerve without interference with the blood supply to the retina.[27]

It was found[15, 16] when the embryonic eyes of salamanders in many stages in development were excised, rotated 180°, and reimplanted, all hosts where the eyes had been rotated before the optic cup stage of development later showed normal vision. However, all hosts whose eyes had been rotated 180° in the early optic cup stage or later showed all visuo-motor responses were reversed. This indicated that in an early stage before the future retina has been differentiated the presumptive functional quadrants of the neural retina are already polarized before the ganglion cells are even differentiated. These studies, which are now in progress, can be extended further, for the embryonic eyes can be exchanged between salamanders of different species so that later a large functional eye on a smaller species (Fig. 9) and vice versa can be studied with respect to visual pattern and the effects upon the organization of the optic tectum.

We know that if the optic cup is removed from its normal environment and placed upon the side of the body of an embryo of the same age, it will complete its development and differentiate during the larval life of the host (Fig. 10). If it is then transplanted to another larval host of the same age and put in place of a functioning eye, the optic nerve will regenerate and eventually connect with the brain and establish vision. In this case, of

course, the retina survives. Normal or reversed vision depends upon whether the transplanted eye is normally oriented or rotated 180°. At this early stage of development the eye can therefore develop into a potential functional eye without continuous connection with the brain.

We have already mentioned that the functional polarized neural retina in the eyes of adult newts degenerate and then regenerate when the eyes are transplanted. Therefore, we shall examine briefly the functional organization of the new neural retina which is regenerated from the surviving single layer of retina pigment cells. This provides an unique opportunity for a variety of studies concerned with the development of the mechanism of vision. Sperry[9-13] has published a number of excellent studies on the functional recovery of vision after the regeneration of the optic nerve in transplanted rotated eyes of amphibians and fishes. References to only a few are included here. Wherein his experiments overlap those of the present author[18, 20] there is good agreement in the results. I shall give here only a brief account of methods and main findings to indicate the part which the regenerating new retina of the salamander eye offers in these studies.

If the eye is excised and reimplanted with normal orientation and the other normal eye is removed, all visuo-motor responses later are normal. The animal moves toward and follows a moving lure, such as a dark-colored bead impaled on the end of a wire, as it approaches the nasal, temporal, dorsal, and ventral quadrants of the fields of vision. However, if the eye is excised, rotated 180°, and then reimplanted, reversing all poles, the visuo-motor responses, as they gradually return, are completely reversed. The animal darts incorrectly in the opposite direction as the lure approaches it. These reactions are the same as in control animals, where the eye is simply rotated 180° *in situ*, and fixed in place after cutting only

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FIG. 1. L2—lens regenerate in accessory opening produced by plastic membrane, M. L1—lens regenerate in primary pupil.

FIG. 2. Regenerated iris from retina pigment cells 103 days after complete iridectomy. x8.

FIG. 3. Normal retina of adult newt eye. x125.

FIG. 4. Complete degeneration of neural retina. Retina pigment cells survive (indicated by arrow), 16 days after eye was excised and reimplanted. x125.

FIG. 5. Complete regeneration of neural retina from retina pigment cells 77 days after eye was grafted. x125.

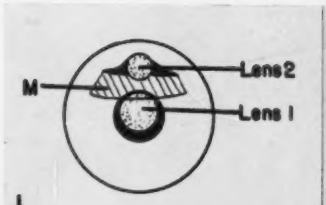
FIG. 6. Transplanted rotated (180°) eye 4½ years after operation. Continuous abnormal vision. x8.

FIG. 7. Normal eye of adult newt. x8.

FIG. 8. Eye 4 months after fourth successful transplantation with return of vision each time. x8.

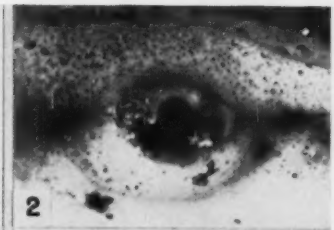
FIG. 9. Young adult *Amblystoma punctatum* host with large right *A. tigrinum* eye which was transplanted 101 days earlier in an embryonic stage of development. x6.

FIG. 10. Salamander larva (*A. punctatum*) with normal developing eye on side of body 10 weeks after optic cup was implanted. x6.



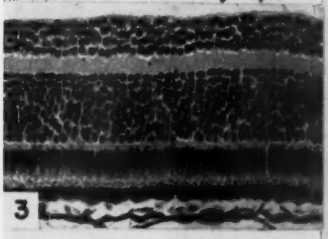
1

Lens 1 In Primary Pupil
Lens 2 In Accessory Pupil



2

Regenerated Iris
103 Days

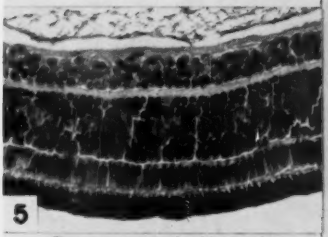


3

Normal Retina

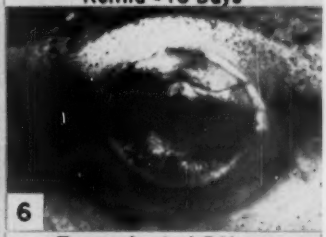
4

Degenerated Neural
Retina - 16 Days



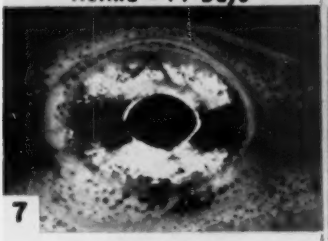
5

Regenerated Neural
Retina - 77 Days



6

Transplant 4.5 Years
Rotated 180°



7

Normal Eye

8

Transplant
4th Time

9

Right Large
Graft - 101 Days

10

Eye On Body
10 Weeks

the conjunctiva and muscle attachments. The blood supply, optic nerve, and original retina are then preserved in the animal so that vision is uninterrupted.

In some cases in which the transplanted, 180°-rotated eye had been functioning abnormally for two years, the eye was excised and reimplanted in the normal orientation. When vision returned again, the visuo-motor responses were normal.

In some animals the reversed vision had persisted for four years and seven months after operation (Fig. 6). In these cases the eye with the regenerated retina was then rotated *in situ* back to normal position without injury to optic nerve or retina. Normal vision was immediately restored.

It was shown that it is possible to study in the same transplanted eye the effect of rotation on two opposite quadrants of the retina, while the other two remained normally oriented. This is accomplished by exchanging right and left eyes with proper orientation. When the retina and optic nerve regenerated, vision was reversed only in those retinal fields which were reversed. These experiments prove that the various functional quadrants of the regenerated retina are always restored, regardless of its orientation. How the regenerated optic nerve fibers reach the proper brain centers to register the position of the retinal field is still unknown.

At present, very little has been done to explore what hidden capacity, if any, the retina pigment cells have in the eyes of many vertebrates at any stage in development. We have in progress a study of this question in the eyes of birds and some of the small mammals. This is a very fruitful field for many who may choose to explore it.

SUMMARY

From the results of these experiments it is obvious that the eyes of salamanders present unique opportunities to study not only the development of the visual mechanism from the embryonic to the adult stage, but the factors which play an important role in regeneration of specialized tissues.

These experiments have demonstrated that in one type of vertebrate eye the presumptive functional fields of the retina become polarized before cellular differentiation takes place and therefore before the optic nerve fibers from the ganglion cells in the retina reach the developing optic tectum. We know that a state of hyperplasia or hypoplasia of the optic tectum can be experimentally produced in salamanders by connecting it with an unusually large or small eye of different species during early development. In this way we can also learn more about the possible influence the eye exerts upon the functional organization of the visual mechanism in the brain.

The eyes of certain salamanders are also remarkable due to the fact that in them the retina pigment epithelium is capable of giving rise to neural retina, iris, and lens tissue under appropriate experimental conditions. It would be interesting to explore from an embryological point of view how this is brought about and whether or not it temporarily exists at any stage of development in other vertebrate eyes.

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EVENTS

PHARMACOLOGY LECTURE

March 17, 1960

THE FUNCTION OF NORADRENALINE PRESENT IN THE HEART AND BLOOD VESSELS. By J. H. Burn, Professor of Pharmacology, Oxford University, Oxford, England.

During the last thirty years, a growing number of observations have suggested that there are qualitative as well as quantitative differences in the activity of various sympathomimetic amines. Carlsson, noting that tyramine failed to show a pressor effect in reserpinized cats, suggested that tyramine was one of a group of amines which required an "intact adrenergic system" for its action. It is known that reserpine pretreatment causes depletion of noradrenaline contained in the walls and chromaffin cells of blood vessels. Tyramine and several other sympathomimetic amines which are not catechol derivatives such as ephedrine, amphetamine, and phenethylamine lose pressor activity as well as the ability to contract spleen and nictitating membrane in reserpinized preparations. Conversely, noradrenaline, adrenaline, dopamine, and phenylephrine have their sympathomimetic actions increased by reserpine pretreatment. When noradrenaline is infused into these reserpine-treated animals, blood pressure rises, then subsides. Tyramine given at this time has its sympathomimetic effect restored, showing that noradrenaline has been taken up from the blood stream into "stores." At this point also, the effects of catechol amines return to the normal range. Observations of this kind suggest that the tyramine group of substances act by the release of noradrenaline from these stores. A small, continuous discharge of this substance is also suggested which acts to diminish sensitivity to injected noradrenaline. A relationship to the hitherto unexplained phenomenon of supersensitivity is apparent.

Such a hypothesis serves to elucidate certain unexplained autonomic effects: nicotine, for example, contracts the nictitating membrane, such contraction not occurring if the animal has been treated with reserpine. Similarly, acetylcholine causes splenic contraction only in the presence of intact sympathetic fibers to the spleen, for if the sympathetic fibers degenerate as a result of section, the store of noradrenaline disappears. Such findings suggest the presence of cholinergic mechanisms operating in the sympathetic supply to release a stored quantity of the traditionally adrenergic transmitter. Are these cholinergic neurones the dark-staining cells seen in the superior cervical ganglion? How is the connection made with chromaffin tissue in the periphery? These and many other questions remain to be answered.

D.M.B.

ZOOLOGY JOURNAL CLUB

March 23, 1960

CONDUCTION IN NONSTRIATED MUSCLE. By C. Ladd Prosser, Department of Physiology, University of Illinois, Urbana.

Studies of nonstriated muscle reveal that the time constants for muscular contraction differ over several orders of magnitude. Histological differences, such as fiber length, are insufficient to account for these differences. Similarly, the slight differences in contractile proteins and metabolism are insufficient. The greatest variations are in the mode of conduction of the contractile stimulus. These are: (1) nervous activation, (2) cell to cell stretch, and (3) cell-to-cell electrical conduction.

Among the invertebrates, only (1) and (2) are found. In the protractor muscle of *Golfingia* two action potentials can be demonstrated, and two types of ramifying nerves have been shown to innervate the length of the muscle. There is no propagation of the impulse in the muscle itself and conduction is solely by means of nervous activation. It can also be shown that the retractor muscle of *Thione* has a nervous basis of conduction. Indeed, it may be generalised that for all postural nonstriated invertebrate muscles, conduction is entirely nervous. The visceral muscles of *Golfingia*, on the other hand, are activated by mechanical stretch, conducted from cell to cell.

Vertebrate nonstriated muscle, or smooth muscle, conducts by electrical potentials between cells. In the 6-12-day chick amnion no nerves have been found which could conduct and in the ureter of the rat nervous impulses move at a different rate than the conduction velocity. Attempts to find a chemical transmitter have failed and evidence of a more general nature upholds the view that chemical activation is not functioning. Electron microscopy has failed to show any protoplasmic continuity between cells. Mechanical stretching also does not activate these muscles. An inverse correlation between speed of conduction and extracellular space supports the conclusion that conduction in vertebrate smooth muscle is by electrical potentials moving from cell to cell. This means of conduction is unique to the vertebrates; thus, the term "smooth muscle" should be applied only to vertebrate nonstriated muscle. True smooth muscle is thought to be an evolutionary invention of the vertebrates alone.

TRACY L. SIMPSON

April 25, 1960

CHICK EPIDERMAL DIFFERENTIATION: *in situ* AND *in vitro* IN CHEMICALLY DEFINED MEDIA. By Norman K. Wessells, Department of Zoology, Yale University.

The differentiation of embryonic chick shank epidermis was studied *in situ* and *in vitro* using chemically defined media. The normal skin was examined with regard to morphology, birefringence, trypsin resistance, SH-SS and RNA distributions, and other histochemical procedures. The

response of cultured skin was investigated in relation to the sulfur-containing components of the nutrient media.

Fibrous elements of the keratin system are present in abundance in the subperidermal cell layer and in some of the outer squamous cells at 16 days *in ovo*. These tonofibrils are birefringent, show resistance to trypsin digestion, and are associated with large quantities of SH groups.

Cornification occurs in the outer regions between days 16 and 17. Nuclei, lateral cell boundaries, tonofibrils, and granular cytoplasmic components all disappear. A glassy matrix with high SS content takes their place.

In a basal medium (derived from Waymouth, 1959), 12-day explants normally develop to the tonofibril 16-day stage. One group in the basal medium and one-third of those explants cultured on raised methionine concentrations underwent cornification. Development to the tonofibril stage was normal when (1) exogenous cysteine and cystine were eliminated, and (2) all major sulfur suppliers, except methionine, were omitted. Quantities of cornified material were reduced in both cases. Methionine, an essential amino acid for the shank skin, could not be replaced with Norleucine or the α-OH analogue. Homocysteine, as a methionine substitute, permitted survival, but development only to the 14-day stage; when serine also was present, some explants formed tonofibrils.

Explants showed normal birefringence, trypsin resistance, and SH content, regardless of the culture medium employed. RNA and SS contents were abnormal.

N. K. W.

May 9, 1960

THE STABILITY *in vitro* OF EMBRYONIC CHICK THYROID CELLS. By S. Robert Hilfer, Department of Zoology, Yale University.

The thyroid was chosen to test stability of morphological and physiological characters *in vitro* because of its distinct characteristics of differentiation—follicular structure, colloid-containing glycoprotein, hormones, and precursors. Glands of 16-day chick embryos were dissociated with trypsin and versene to destroy tissue organization. Cultures were prepared by reaggregating the cell suspensions thus produced and culturing on lens paper rafts at the air-medium interface, or by further disorganizing the cells in spreading culture before reaggregating and placing in organ-type culture. Glycoprotein was detected with the periodic acid Schiff reaction and the iodinated compounds were separated by standard chromatographic procedures and visualized with the ceric sulfate arsenious acid reagent.

New follicles containing glycoprotein appeared in aggregates of freshly dissociated cells; these cultures contained thyroxin and its precursors. During spreading, on the other hand, the precursors and glycoprotein were still contained within the cells, but thyroxin was lost and no follicles formed. After reaggregation and organ culture, the cultures still contained no follicles or hormone. TSH treatment had no visible effect on these two

characters but did increase the rate of glycoprotein formation in both types of aggregates. When fibroblast cultures from thyroid capsule were reaggregated in combination with spreading epithelium, however, large amounts of extracellular colloid and some follicles appeared, but thyroxin was still absent.

N. K. W.

May 16, 1960

STUDIES ON MORPHOGENESIS IN A CILIATE, *Glaucoma chattoni*. By Joseph Frankel, Department of Zoology, Yale University.

The techniques of silver impregnation and of digitonin solubilization have been applied to an investigation of aspects of cortical morphogenesis in *Glaucoma chattoni*. The typical stages of the formation of new oral areas (stomatogenesis), as well as of other related surface events, are described in detail. Estimates were made of the relative and absolute durations of these stages; these estimates indicate that for animals growing at 25° C. in bacterized culture, stomatogenesis and cell division together occupy about one-third of the division cycle. The process of increase in number of kinetosomes along certain kineties has been submitted to extensive study. The bulk of this increase occurs not during the period of stomatogenesis and cell division, but rather during the remaining two-thirds of the division cycle. Data relevant to this increase reveal that the probable phases of most rapid kinetosomal increase, along kinety 1 at least, occur just after division and just before the beginning of stomatogenesis.

Individuals of *Glaucoma chattoni* were immobilized in a microcompression chamber, and their oral areas were irradiated with an ultraviolet microbeam. The damaged oral areas that resulted gradually disappeared, and were replaced by the differentiation of a new oral primordium. This process could be made to take place at all parts of the division cycle. It could also be provoked several times in succession in the same animal. In dividing animals, severe compression alone was sufficient to damage the existing oral area and stimulate oral replacement.

Study with the digitonin solubilization method revealed that new regeneration primordia appear at the anterior end of kinety 1 and follow a pattern of differentiation identical to that which occurs during the formation of new oral areas prior to division.

Irradiation of oral areas of dividing animals or of animals just about to begin division failed to prevent division from going to completion, while irradiation at a slightly earlier stage frequently induced fisson blocks such that if a division furrow formed, it subsequently regressed. Following such regression, animals came to possess a single macronucleus and either one or two sets of cytoplasmic organelles.

N. K. W.

May 20, 1960

THE DEVELOPMENTAL PHYSIOLOGY OF A FEMALE STERILITY MUTANT IN *Drosophila*. By Winifred Doane, Department of Zoology, Yale University.

An analysis was made of relationships between developmental and physiological characteristics of the recessive mutant *female sterile (2) adipose,* located at 2.83± on Chromosome II of *Drosophila melanogaster.* Controlled homozygous and heterozygus genetic backgrounds were used as an experimental technique. Cytological abnormalities in eggs laid by homozygotes are described, including a maternal age effect that superimposes a "yolk-deficiency syndrome" on a basic cytoplasmic cause of lethality. This maternal effect is eliminated in one of the genetic backgrounds, along with reduced longevity and fecundity typical of mated *adp/adp* females.

Larval manifestations include prolonged development, storage of excess reserves, and an effect on ovarian size. Homozygotes, and to a lesser degree heterozygotes, are characterized by enlarged adult fat bodies. Homozygotes store almost twice as much fat as normal and iodine number determinations show greater saturation in their total body lipids. Larval and adult transplantations indicate that normal ovarian implants inhibit fat storage by *adipose* females but mutant implants do not. Implanted corpus allatum-complexes do not alleviate the mutant fat condition, and injury tends to exaggerate it. The corpus allatum hypertrophies in mated *adipose* females but not in virgins or males. This condition is unstable and alleviated by implanted wild type ovaries or by injection of Ringer solution into adults. Effects of age, mating, starvation, and other factors on adult corpus allatum size were analyzed in mutant and wild types. Sterility of *adipose* ovaries is autonomous and wild type ovaries develop normally in mutant hosts. A theory is presented that suggests a hormone released by normal ovaries partially regulates fat utilization in conjunction with reproductive activities of females. This hormone, deficient in *adipose,* may also influence corpus allatum activity.

Adipose adults utilize their fat during starvation, affording a selective advantage to heterozygotes and homozygotes. A selective advantage under desiccation is displayed by heterozygotes. Evidence suggests that *adipose* persists in natural populations in a balanced polymorphic state.

WINIFRED DOANE

PATHOLOGY SEMINAR

May 19, 1960

SYNTHESIS OF COLLAGEN. By Gabriel C. Godman, Columbia University.

Studies employing tissue culture techniques and electron microscopy throw new light on the origin of the intercellular matrix of connective tissues. In the former experiments, fibroblasts from a variety of sources, when cultured under constant growth-promoting conditions, produced large quantities of acid mucopolysaccharides; all cell populations manufactured

the same molecular pattern (*ca.* 70 per cent hyaluronic acid, less condroitin sulfate). These same fibroblasts *in situ* would each have produced a different characteristic pattern, and one that would vary with the stage of tissue differentiation. With nongrowth promoting conditions the specific production of the acid mucopolysaccharides fell, thus indicating that population growth and AMPS production are directly related.

Electron micrographs of developing rat cartilage cells indicate that both periodic fibrous structures (*ca.* 80 Å repeat distance) and various nonfibrous inclusions of the cartilage matrix are derived from intracellular constituents. During ontogeny, as the intercellular clefts increase in area and the endoplasmic reticulum and Golgi apparatus become definitive in the cells, electron dense areas can be found both within the surfaces of the chondroblasts and extending out into the matrix. The areas are composed of parallel arrays of filaments (60-80 Å diam.), some of which display 80 Å periodicity. These filaments are thought to give rise to the matrix fibers (*ca.* 150 Å diam.), perhaps by acting as accretion centers for tropocollagen molecules.

Within the chondroblasts are large, clear "pools" that are surrounded by double membranes. Inside these clear areas are found amorphous or granular inclusions, occasional mitochondria, or endoplasmic reticulum-type vesicles. All of these entities may be discharged into the cartilage matrix via large holes that apparently develop in the cell surface. Similar pools, sometimes in the process of discharging, may be discernible in mature chondrocytes.

N. K. W.

BOOK REVIEWS

THE CLONAL SELECTION THEORY OF ACQUIRED IMMUNITY. By Sir Macfarlane Burnet. Nashville, Vanderbilt University Press, 1959. xiii, 209 pp. $5.00.

Drawing upon our knowledge of microbial genetics, Burnet has proposed a theory of antibody formation which offers an explanation for certain poorly understood immunologic phenomena (for example, the persistence of antibody for long periods in the absence of contact with the antigen, and the failure to form antibodies to self-antigens under normal conditions). According to Burnet's hypothesis, antibody-producing cells are a mobile population constantly undergoing physiological and mutational change. When mutation occurs, a new clone of cells capable of producing only one kind of antibody is created. It is assumed that all antibody is a consequence of such mutation. Contact by a clone with the antigenic determinant is stimulatory and may lead to a proliferative response with selective advantage to the clone. Under certain circumstances, as in embryonic life, contact by the clone with the antigen may lead to the death of the clone. As a result, antibodies against self cannot be produced unless a similar clone subsequently arises by mutation.

This book is devoted to reviewing the information leading to and supporting this theory and to developing its corollaries. The author admits the theory will undoubtedly require revision. There is an extensive and stimulating discussion of its relation to such subjects as natural antibody, immunologic tolerance, homograft immunity, thyroiditis, collagen disease, sarcoid, agammaglobulinemia, leukemia, cancer, and the course of certain infectious diseases.

It is a scholarly and well-written book which could be read profitably by anyone interested in cellular biology, immunology, or infectious disease.

F. ROBERT FEKETY

PREVENTIVE MEDICINE: PRINCIPLES OF PREVENTION IN OCCURRENCE AND PROGRESSION OF DISEASE. Edited by Herman E. Hilleboe and Granville W. Larimore. Philadelphia and London, W. B. Saunders Company, 1959. 731 pp. $12.00.

The editors of this textbook, Drs. Hilleboe and Larimore, are respectively the Commissioner and Deputy Commissioner of the Health Department of the State of New York. The remaining 29 contributors are either presently or have been previously associated with the organization. The subject is covered in 41 signed chapters which are organized in three general areas: Part I. Prevention of Occurrence; Part II. Prevention of Progression; and Part III. Supporting Services for Preventive Medicine. The scope of the coverage ranges from presentations of the classic public health and preventive medicine responsibilities of sanitation of the environment and com-

municable disease control to the newer responsibilities of the hazards of ionizing radiation and medical defense against atomic attack.

The individual topics are adequately developed with emphasis, in the majority, on brevity of presentation rather than complete and exhaustive detail. References are listed after each chapter for the reader desiring more definitive information. "Epidemiologic Methods and Inferences" by Dr. Dienfeld and "Official and Voluntary Health Agencies" by Dr. Hilleboe are two chapters which offer especially well-organized, succinct, and effective discussions of their respective subjects.

The editors state in their preface that they are presenting ". . . a new way to look at preventive medicine for the medical students, general practitioners, specialists, and professional workers in official and voluntary health agencies." In the opinion of the reviewer, the authors have achieved their purpose by editing a book which is more an introductory text than a reference tome.

WILLIAM L. KISSICK

HISTOCHEMISTRY, THEORETICAL AND APPLIED. 2d ed. By A. G. Everson Pearse. Boston, Little, Brown and Company, 1960. 998 pp. $20.00.

This new work by A. G. Everson Pearse attests to the fact that the subject of histo- and cytochemistry has developed with both rapidity and clarity. Rarely in the history of the basic medical sciences has a subject expanded like histochemistry. The first edition published a little over five years ago contained only 500 pages; the present work is not only double in size, but includes numerous and well-selected illustrations of several of the techniques described. In comparing it with its predecessor, I was immediately impressed with the fact that histochemistry has been made easy because of the clarity of expression of the author and the numerous simplifications and modifications of previously described methods.

The present volume is free of tacit assumptions. The opening chapter is devoted to a history of the subject and proceeds to a discussion of cold knife and cold microtome methods, freeze-drying and freeze-substitution, and the chemistry of fixation. The succeeding chapters, for the most part, deal with the practical application of diagnostic and analytic methods, both chemical and physical, to tissue sections. Of great advantage to the workers using this book are the numerous details and critical comments which are provided for clarity and ready use of the methods cited.

The section on enzyme histochemistry has been greatly expanded. This is certainly warranted, especially since there are techniques now to demonstrate 45 enzymes (in contrast to 3 in 1951 and 18 in 1953). Furthermore, the rapid advances in enzyme histochemistry were accompanied by rather detailed refinements and improvements in the earlier described techniques. Up to 1953 most of the techniques were concerned with the hydrolytic enzymes, mainly phosphatases and esterases. No methods existed for the different amino-acid oxidases, urease, arginase, or peptidases. Methods are now available for the demonstration of D-amino oxidase and leucine amino-

peptidase, while enzymes of the cathepsin class can be demonstrated by a combination of existing methods with suitable activators and inhibitors.

Another valuable feature of this book is the detailed appendices on the different methods of preparing tissues. Each is written in a careful step-by-step fashion and contains preparations, methods, and result. Also, pertinent and extensive bibliographies accompany each chapter.

The book is produced on good paper, the illustrative material appears on heavier stock, and the type is of good size, clear, and luxuriously spaced, all of which facilitates reading.

JOSEPH THOMAS VELARDO

LIPIDE CHEMISTRY. By Donald J. Hanahan, with contributing chapters by Frank R. N. Gurd and Irving Zabin. New York, John Wiley and Sons, Inc., 1960. 330 pp. $10.00.

This comprehensive survey of the more recent work on the chemistry of lipides—and, especially, of the newer techniques used in the study of these substances—is a most welcome addition to the biochemical literature. As Dr. Hanahan remarks in his Preface, the current interest in lipides "is due in no small part to the great emphasis placed on the etiology and the possible cures for atherosclerosis and other related lipide diseases," and a knowledge of the structure of those naturally occurring compounds grouped together under the heading "fats" or "lipides" is important not only to the biochemist and physiologist but also to the clinician.

This book is concerned with the substances classified as simple lipides (i.e., esters of fatty acids and an alcohol such as glycerol or cholesterol), phospholipides (i.e., phospholipides containing glycerol or sphingosine), and lipoproteins found in mammals, plants, and microorganisms, with emphasis placed mainly on the substances of mammalian origin. For each class of lipide there is presented a thorough and critical discussion of the methods of isolation and the procedures used to establish the purity and structure of the isolated material. The description of the current status of knowledge concerning the plasmalogens (acetal phospholipides) is especially noteworthy, as is Dr. Zabin's chapter on the sphingolipides termed sphingomyelins, cerebrosides, and "mucolipids" (gangliosides, strandin, etc.) The complicated subject of lipoprotein chemistry is summarized in two excellent chapters by Dr. Gurd, one of which deals with the interaction of lipides and proteins and the other with naturally occurring lipoprotein systems. Each chapter in the book has a list of references that includes experimental papers (a few published as recently as the early part of 1959), review articles, and monographs. Although greatest emphasis is placed on advances made in the last 10 or 15 years, and the reader must go elsewhere for a detailed discussion of the early work, direct references to all the major discoveries, regardless of date, are included in the bibliographies.

It is to be regretted that a book which undoubtedly will be used for reference by students and more advanced investigators alike does not have a more complete index. For example, the index lacks specific entries for

bile acids, ceramide, chylomicrons, gangliosides, and lecithin—all of which are considered in the text.

SOFIA SIMMONDS

A GUIDE-BOOK TO BIOCHEMISTRY. By K. Harrison. New York, Cambridge University Press, 1959. viii, 150 pp. $3.50.

An offer of guidance through biochemistry in the space of 150 pages cannot fail to evoke an eager response. We must agree with the author as to the need, as an introduction, for something less formidable than most of the current textbooks. This little book offers us, in the author's words, the bare bones, and is intended for students in a first biochemistry course and for those wondering whether to take such a course.

The plan of the volume is admirable: an introductory chapter, followed by one on energy changes in biochemical reactions, one on enzymes as proteins and as catalysts, one on biological oxidation, then four chapters on various branches of metabolism, and finally a short discussion of control mechanisms in metabolism. The earlier chapters are perhaps the most satisfactory, with a healthy emphasis on principles and adequate coverage of empirical fact. Consideration of metabolism begins with a rather detailed discussion of the pathways of carbon in photosynthesis, and one may question whether it would not have been safer to start the student off with something less complex and controversial. The allotment of space to different subjects is also unusual: 15 pages are devoted to photosynthesis and 6 to the pentose phosphate pathway, while nucleic acids are dismissed in a page and a half and phospholipids with the mere statement that "we must not stop here to consider them."

It seems to the reviewer that the skeleton presented is often so bare as to be dry, and might better hold the student's interest if filled out with more of the substance of experimental observation. Thus, we are told that a typical enzyme has a relatively small number of active centers, but we are given no indication of the evidence for this. Again, Knoop is cited as having proved that fatty acids are broken down two carbons at a time, but no space is found to describe the classic and prophetic experiments that led him to this conclusion. A brief discussion of the Michaelis-Menten theory, including a definition of the K_m, is presented without the aid of a single graph. These are but examples of the rather abstract plane of presentation which is used throughout. The book has very few figures, and it seems doubtful whether this feature, doubtless aimed at economy of production, is worth the sacrifice of clarity.

Some of the conventions employed are not those in widest use at the present time, and perhaps not the most helpful. For example, reduced diphosphopyridine nucleotide is designated as $DPNH_2$, and the rH scale is preferred to the use of oxidation-reduction potentials. There are also a certain number of statements which seem dated or inaccurate, but this is understandable in view of the virtual impossibility of keeping up with developments in all branches of biochemistry at the present time. Possibly Dr. Harrison has attempted to compress rather too much into a volume of this

size, and his work may prove difficult for the beginning students at whom it is aimed. It will, however, assist those who have already picked up some fragmentary knowledge of the subject in organizing their ideas into a logical whole.

G. R. WYATT

GLUTATHIONE. E. M. Cook, Ed. Biochemical Society Symposium No. 17 held at Senate House, University of London, 15 February 1958. Cambridge, Cambridge University Press, 1959. 115 pp. $4.50.

The 1958 symposium of the Biochemical Society consists of seven papers given by British biochemists on areas of glutathione (GSH) chemistry and biochemistry. The topics presented may be listed briefly: chemistry of GSH, techniques for determining GSH, oxidation-reduction systems associated with GSH in plant tissues, GSH in animal tissues, and neural tissues, and in lens, and finally the effect of thiols in radiation damage.

On the whole, it may be stated that the topics are covered concisely and critically. The earlier book *Glutathione,* published in 1953, is much more valuable due to greater coverage and open discussions which are not part of this later book. Much of the material presented in the earlier book is inevitably repeated since the progress in fields relating to the chemistry and role of glutathione has not been extensive. Unique contributions of this volume lie in the chapter by Mapson in which the work bearing on the question of the role of GSH oxidation-reduction in terminal electron transport in plants is covered and evaluated and in the chapter by Waley in which the occurrence and role of GSH and its analogues in the eye lens are considered. Since conjecture on the role of GSH in natural systems has emphasized the -SH group, it is of particular interest to note that opthalmic acid (ó-glutamyl-α-amino-n-butyrylglycine) is as effective as GSH in triggering the "feeding reaction" of hydra.

IRWIN A. ROSE

BIOPHYSICAL SCIENCE. A Study Program planned and edited by J. L. Oncley *et al.* New York, John Wiley & Sons, 1959. 568 pp. $6.50.

This volume reports on the formal part of a program arranged by the Biophysics and Biophysical Study Section (of the National Institutes of Health) which took place at Boulder, Colorado in the summer of 1958; its purpose was to bring together an active group of scientists from different fields who have been endeavoring to apply the fundamentals and techniques of chemistry and physics in resolving basic problems in the life sciences.

As a framework to provide the nexus between interactions within and between cells (and in tissue fabrics), the first quarter of the book discusses the chemistry and physics of biological macromolecules and their component parts. The very recent important advances in our knowledge of the three dimensional organization of polypeptides and proteins, polynucleotides and nucleic acids are well documented. After many decades of effort a model of a protein molecule, myoglobin, based on analysis x-ray data, with resolution

to 6Å has been worked out. The existence of helical structure in this globular protein, which is formed from the polypeptide backbone, appears to be unambiguously confirmed. The behavior of polymers in dilute and concentrated solutions and one-dimensional transitions in synthetic polypeptides and polynucleotides are reviewed by several authors.

The second quarter of the volume is concerned principally with the dynamics of cellular respiration, bio- and photosynthetic processes. The central role of enzymes as biocatalysts is evident throughout. The recent success in the synthesis of desoxyribonucleic acid and polyribonucleotides is reviewed. The ability to synthesize simpler analogues of the nucleic acids and to study their behavior and interactions is now feasible. Although the *in vitro* synthesis of a protein, or even a long chain polypeptide has not been achieved as yet, recent progress in this classical search makes this development appear close to fruition. To emphasize the significance of the role of proteins and nucleic acids several articles are presented on the function of nucleic acid (DNA) as genetic determinants, their replication in several cellular systems and coding mechanisms based on the nucleotide sequence in DNA.

Several chapters deal with the effects of high energy elementary particles and x-irradiation on the physical and biological parameters of macromolecules, viruses, and cells. Relationships between radiation dosages and loss of various biological activities, mutation and carcinogenesis are discussed.

The last half of the volume largely describes the major macroscopic intracellular organelles and the membranous filaments which tend to organize cells into tissues. Many of the developments in this area have accrued from newer refinements in electron microscopic techniques although x-ray and classical optical methods are frequently used conjointly. The interactions of collagen with itself and the influence of other molecules on its ability to reform ordered aggregates possessing structural integrity are reviewed. The formation of various types of periodic collagenous assemblies provides significant clues as to how properties of individual protein molecules may be incorporated into higher organized structures. Less success has been encountered in reconstituting muscle from its constituent extractable proteins, which individually have been extensively characterized. Similarly, the mechanism of muscular contraction is still largely speculative.

The correlation of anatomical fine structure with physiological function is elaborated upon by a group of reports on the ultrastructure of nerve fibers, ion transport across nerve membranes, and the mechanism of formation and propagation of nerve impulses. Discussion of still higher levels of organismic coordination tabulated under the phenomenon of biological transduction is also presented.

It is apparent from the cursory description provided in this review of the various areas of biochemical and biophysical endeavor discussed in this volume that the scope is indeed broad; simultaneously many of the articles provide comprehensive analyses and speculation of the significance of the new developments they have outlined.

The reviewer has refrained from singling out particular chapters and authors for commendation since few would not warrant special approbation.

The many achievements ensuing in the past decade from the interdisciplinary approaches employed are very tangible in this volume. It is almost a truism these days to say that much that is original and of far-reaching consequence in contemporary biology is the result of the application of the concepts and particularly the techniques of chemistry and physics. The converse proposition is equally appropriate, at least with respect to chemistry, for it has been stimulated and enriched by the solution of problems emanating from biological systems. There are also many intimations in this volume that the skills and insights of the theoretical physicist and mathematician will be needed to develop adequately many problems in biology now only at their threshold.

The 61 articles contained in this volume are generally reviews of a specific area of investigation and are presented at a graduate level of sophistication. On the other hand, they may be read with much profit by people with scientific backgrounds who possess sufficient interest. The text as a whole presents very successfully the broad panorama of the interplay and diffusion of physico-chemical science to biology. The Study Section and the Editorial Board are to be congratulated for bringing to the scientific community, in a single volume, at an exceptionally modest price, this superb monograph.

HAROLD EDELHOCH

MICROBIOLOGY. By Louis P. Gebhardt and Dean A. Anderson. 2d ed. St. Louis, C. V. Mosby Co., 1959. 476 pp. $5.75.

This book deserves to be criticized severely on a number of levels. The authors state that the text is intended to "present the basic concepts of microbiology in a manner understandable to the general student who lacks extensive background in the biological and physical sciences." However, it appears to be aimed primarily at students who plan to enter technical careers requiring some understanding of microbiology. Approximately one-third of the book is devoted to general microbiology, one-third to "Sanitary and Industrial Microbiology," and one-third to pathogenic microbiology.

My major criticism is that the basic concepts of microbiology are not presented clearly. The most consistent defect of microbiology are not presented clearly. The most consistent defect of the presentation is a general ambiguity and lack of conciseness. In addition, there are numerous misleading oversimplifications, half-explanations, and over-extensions of analogy. There is no obvious attempt at an over-all logical development and little, if any, evidence is presented for the generalizations made.

No coherent effort is made to provide even the simplest background of biological and biochemical thought. Nevertheless, the authors refer to sophisticated concepts of biochemistry and genetics. They use the symbol DNA with great abandon although neither DNA nor deoxyribonucleic acid appear in the index or the glossary.

The writing is careless and awkward. It contains popular slang and unnecessary technical jargon. The condescending style and folksy humor would, I think, offend any student of average intelligence.

The illustrations are appalling. The drawings are for the most part so crude and inaccurate as to be scarcely acceptable from a beginning biology student. Many of the photographs have no educational value whatsoever. There are numerous photographs of large machines and of people performing various operations in laboratories. Some photographs that might be instructive are rendered useless by poor reproduction and faulty labeling.

The book presents a lot of watered-down, uncorrelated information in a confusing and careless manner with little serious effort to explain it to the student or to give any idea of the experimental basis for modern concepts in the field.

It would be a great misfortune if a technical student assumed he had mastered the subject after being exposed to this text. Any serious inquiring student would be discouraged, I think, from further exploration in microbiology by this presentation.

BARBARA J. BACHMAN

GENETICS AND CANCER. A Collection of Papers Presented at the Thirteenth Annual Symposium on Fundamental Cancer Research, 1959, University of Texas, M. D. Anderson Hospital. Austin, University of Texas Press, 1959. 459 pp. $8.50.

This well-bound and nicely printed volume is excellent. It is timely. The contributions of the microbiologist in biochemical genetics, of those exploiting tissue culture technics, the immunologist, the electron microscopist, the virologist and mammalian geneticists, as well as those concerned with "inborn errors" in man and more refined epidemiological studies, are all focusing increasingly on basic problems that may contribute very significantly to a greater understanding of cancer. All these aspects are reviewed in relation to cancer in this volume in a concise and reasonably critical manner. In most instances the authors have selected references critically, have drawn extensively from their own observations, and have been uniquely lucid. This must have been a most interesting symposium.

The book is divided into five sections. The first section covers "Genetic Theory of Cancer Etiology." Professor Darlington provocatively presented selected experiments to reinforce "the view that tumor agents always arise from plasmagenes whether or not they are transmissable as proviruses." The demonstrated chromosomal heterogenecity of many tumors indicated that nuclear factors were not so important in tumors. The possibility of somatic mutations in carcinogenesis was discussed by Jack Schultz who pondered the difficulties of accounting for the regularities of cellular differentiation on a mutational basis and states that, "My own view is that a reasonable possibility which does not involve special somatic mutations in the primary initiation of carcinogenesis may be found," but then later states, "the change of cell type in tumor formation involves a change in cell heredity," and "one can hardly escape some form of somatic mutation hypothesis of tumor origin."

The third chapter is by Wollman and Jacob on lysogeny, transduction, and cancer genetics. A brief summary of some of the observations on these

subjects, on the development of the episome theory, and demonstration of colicinogenic factors are presented as they may be extrapolated to implicate viral and mutational changes in carcinogenesis.

The second part of the book covers "*Fundamental Aspects of Genetics in Carcinogenesis.*" The first section by Dr. Saul Kit presents studies on the comparative composition of the deoxyribonucleic acids of different normal and neoplastic tissues. Little difference existed between the two. The attempts to concentrate and identify the agent causing mammary cancer in mice were summarized by Dr. Dmochowski, *et al.* The virus was studied both chemically and by the electron microscope. Virus activity was in RNA fractions and partially inactivated when incubated with chymotrypsin but only slightly affected by trypsin. The particulate materials demonstrated in the different concentrates could be correlated with biological activity. Dr. Latarjet, in his chapter, develops the hypothesis that cancers are evoked by mutations that result in inactivation of a repressor gene in a cell (gene that limits cell growth) and a general alteration of adjacent tissues to prevent diffusion of repressive substances to the altered cell. In this connection he discusses the actions of x-rays, ultraviolet irradiation, and viruses on carcinogenesis and in relation to genetic change.

The influence of added purines, pyrimidines, or their ribosides increased mutations in *E. coli* exposed to ultraviolet light. The mutation inhibiting effects of chloramphenicol were prevented by the addition of amino acids. These and other substances, some of which may arise intrinsically, were discussed by Haas and Dowdney in their chapter in relation to both mutations and carcinogenesis.

Albert Levan tabulated the data on over 300 different chromosome number distributions on normal and malignant tissues from different tissues and organs of animals of several species. The stability of the normal diploid number in normal tissues was striking, especially in stem-line tissues. Variability of chromosome numbers increased with growth *in vitro*. Tumors in general had less constant chromosome numbers and were amenable to selection of clonal variants. The adaptibility of cells from one of the L sublines to carbon sources of different sources was reported by Hsu and Kellog. The chromosome numbers of the variants were also studied.

The section on Gene Interaction and Neoplastic Growth was opened by D. M. Bonner who discussed gene action in sequential quantitative and qualitative regulatory mechanisms. Heston listed 18 genetically determined tumors in mice and discussed experiments that demonstrated linkage groups, probable sites of gene action in carcinogenesis and tumor growth, and factors influencing growth of transplanted tumors.

The use of tumors arising in heterozygous mice (F_1) and in homozygous mice, studied with respect to content of histocompatability genes or alleles, was reported by Dr. G. Klein. Variants were obtained by transferring the hybrid tumors to parental and other hybrid groups of one or the other parental strain. The selection of variants was compared to tumor progression. Dr. Haushka and associates summarized the experiments on the Y-chromosome histocompatability gene as studied using skin grafts and transplanted tumors. Skin and tumors of males failed often to grow in

females when the Y-antigen was present. The rather extensive recent data on the Y-antigen is reviewed. The possibility of a chromosomal abnormality being associated with the development of drug resistance (a-methopteran) was reported by Bieselle, *et al.*

The Genetic Basis of Cell Resistance was discussed in the three papers presented in this section. The relations of hormones and viruses in the progression of mammary lesions in mice, with much new data, were presented by Dr. DeOme. Dr. Woolley discussed the genetic interpretation of the acquired resistance to certain chemicals such as amethopterin—evolutional adaptation is the central theme for explanation.

Bovine ocular carcinoma in Hereford cattle increased progressively with age, was higher among offspring of diseased parents, and also related to periocular pigmentation (Anderson, *et al.*).

The final section on Heredity and Human Cancer was introduced by a paper on recent developments in human genetics by W. J. Shull. Both advances in content and in methods were discussed and included the challenging statement that the present approach cannot advance understanding of the origins of the more common cancers. N. E. Morton discussed methods of studying in human genetics with great anticipation for future accomplishment, and Dr. Macklin and Dr. Oliver presented their well-planned studies on the evidence of partial genetic involvement in human breast, gastric, and rectal cancer.

The Bertner Foundation Lecture by Dr. Richard E. Shope was on the role of the virus and host in determining the host response to the fibroma-myxoma virus complex. Both dynamic variables influence the clinical picture of the disease. At least four related viral entities vary in the responses manifest in diverse hosts.

WILLIAM U. GARDNER

PSYCHIATRY IN THE MEDICAL SPECIALTIES. By Flanders Dunbar. New York, McGraw-Hill, Inc., 1959. 535 pp. $12.

In *Psychiatry in the Medical Specialties* Flanders Dunbar outlines and illustrates her views on the contribution of psychiatry to the diagnosis and treatment of a great many diseases encountered frequently by various specialists. She discusses not just the diseases traditionally viewed as psychiatric and psychosomatic, such as psychosis, neurosis, behavioral disorders, ulcers, hypertension, ulcerative colitis, obesity, but also such diverse disease syndromes as accidents, fractures, coronary artery disease, rheumatic fever and rheumatoid arthritis, diabetes, neoplasms, dental syndromes, tuberculosis, allergy, genito-urinary syndromes, eye and ear syndromes, and old age. The tremendous range of the different types of disease discussed reflect the breadth of the author's interests, as does her distinguished career.

Flanders Dunbar earned a Ph.D. from Columbia (in literature) and a B.D. from the Union Theological Seminary and an M.D. from Yale. She has done clinical research at the New Haven Hospital, Bellevue, Worcester State Hospital, the General and Psychiatric-Neurological Hospital and Clinic of the University of Vienna, the Berghölzli, Zürich, and the Presbyterian Hospital, New York. She was active in the founding of the journal

Psychosomatic Medicine, of which she was the first Editor-in-chief, and in the formation of the American Psychosomatic Society. Her death in 1959 was a great loss to American medicine.

Her best known research is the psychiatric study of serial admissions of patients with a number of prevalent diseases to the Presbyterian Hospital. She gathered information about the patient's personality and environment and formulated for each disease syndrome an associated personality constellation. Flanders Dunbar rejects the notion that each disease has a single biochemical or mechanical cause. She feels that diseases are the results of multiple events, including factors relating to personality and environment, as well as organic factors. The basic assumption of her research is that certain diseases are associated with a certain personality constellation. In *Psychiatry in the Medical Specialties* she outlines the personality constellation for a great many disease syndromes encountered in a wide variety of specialties. The existence of personality constellations associated with different disease syndromes has long been a controversial point in psychosomatic medicine. Many psychoanalysists doubt the existence of personality constellations associated with specific diseases. The crucial evidence has been provided by Ring and is discussed in detail by Flanders Dunbar. Ring conducted psychiatric interviews with a number of patients suffering from a wide variety of medical diseases. He was not given any of the data relating to the medical history or physical exam and all cases in which the patient inadvertently volunteered such data were discarded. From the psychiatric interview alone he was able to make the correct diagnosis in 75 per cent of the subjects. This certainly suggests that certain diseases go with certain personalities, supporting Flanders Dunbar's basic assumption. The usefulness of the personality constellation extends not only to diagnosis of full-blown diseases but in the diagnosis of early stages of diseases which may progress in a patient with a certain personality constellation, thus enabling the wise physician to intervene in the complex change of causal and perhaps irreversible factors before the condition became serious. This would contribute to the practice of a truly preventitive medicine. For example, the physician, seeing a patient of the personality constellation of accident proneness for whatever reason, might take steps in interviewing in this personality predisposition, thus preventing future accidents as well as helping the patient with the present acute problem. Following the discussion of the personality configuration associated with the various diseases discussed Flanders Dunbar provided a section on "Practical Suggestions" for the management of patients.

In addition to the discussion of the personality correlates and psychological management of patients with a wide variety of diseases, Flanders Dunbar provides a comprehensive and interesting discussion of the pathological psycho-physiology and psycho-endocrinology of many of the diseases. In discussing the mechanisms involved she draws information from a wide variety of sources; from psychology and psychoanalysis to endocrinology and physiology, from Voodoo death to cybernetics, information theory, and "operations" research. Her bibliography lists 702 references. One of the great strengths of this book lies in the great amount, breadth, and variety of

the concepts and information it presents. Its great weakness lies in the lack of critical appraisal of the methodology of some of the data quoted. Wide-eyed speculation based on single cases are quoted with as much authority as the data of well-controlled experimental studies.

Considering the fact that at least 65 per cent of patients coming to the average physician have disease syndromes initiated or seriously complicated by psychological factors, and that the busy specialist or general practitioner has had little time to keep in touch with the latest research in psychiatry, this book will be a welcome addition to be read with profit by most medical specialists.

JOHN M. DAVIS

PSYCHIATRY IN MEDICAL PRACTICE. By W. Lindesay Neustatter. London, Staples Press, 1958. 311 pp. $8.00.

The major purpose of this book, as stated in the preface, is to describe various specialized treatments and procedures of modern psychiatry, "primarily for the benefit of those in general practice who are interested in (psychiatric) problems that beset them in their daily work." The book turns out to be a thinned-down standard psychiatric text, based almost entirely on information and opinions at least 15 years old. Although there is refreshing literary simplicity in the book's language and style, its brevity and incompleteness eliminates most of what value the book would have had as a reference text.

Some of the chapters are written by four contributors. Of these, Partridge's on cerebral surgery is a creditable abbreviated review of a very confused treatment area, and Allen's on hypnosis is a gem-like piece of succinct lay coverage of the field of hypnosis and hypnotherapy. What is promised and not given, is adequate treatment of psychological problems that commonly arise in medical practice. For example, there is only a brief paragraph which merely generalizes some aspects of psychological management of acute psychiatric emergencies. There is no mention of postoperative and incapacitating disease states, or consideration of the management of the dying patient. The only discussion of professional control (as opposed to amateur) in the doctor-patient relationship, comes briefly under the heading of "technique of history-taking." What, for instance, should a doctor consider in planning his care of a patient who asks penetrating questions about diagnosis? How do various categories of illness that are described in the book, influence the management say, of an acute infectious illness? How does a doctor cope with strong undesired emotional feelings that arise in himself during the course of patient management? There is little of value to a physician seeking general information which will help in the management of psychological problems.

M. L. PILOT

THE LANGUAGE OF PSYCHOLOGY. By George Mandler and William Kessen. New York, John Wiley & Sons, Inc., 1959. 301 pp. $6.75.

George Mandler and William Kessen, two Yale-trained psychologists, attempt, in this book, a systematic analysis of the language of psychology.

Briefly they argue as follows: The language of psychology begins with the everyday language; however, this everyday vernacular has certain limitations. For example, there is a tendency to reification where words are used as if words and things were interchangeable. In addition there is a tendency to vagueness due to the variable conditions of the learning of the meaning of words. The authors also point out the confusion between man as the object of scientific enquiry and man as the investigator. They argue that the phenomenological language, a strictly physicalistic language or a common-sense thing—language fails to satisfy the criterion for a more scientific language—namely, that its words, whatever their source, must show a high consistency of uses between investigators and over time. They specify that response invariance is the basic criterion for acceptability of verbal responses to environmental events. While rejecting the rigid physicallistic reductionalism, they point out that a cautious physicalism can provide useful words for a language of psychology. The role of definition of terms is discussed, including the difficulty of defining new words with old words whose uses are not sufficiently precise. They argue that operational definitions are useful in certain but not all situations and for the utility of partial reduction statements.

Psychological theories are felt to be sets of statements understandable to others which make predictions about empirical events. It is pointed out that inductive inference, in psychology as in all human activity, takes place in a context of presupposition, both logical and psychological. They discuss the usefulness of well worked out deductive systems and the utility of plausible theories. The psychology of creativity, concept formation, and induction are discussed as are the problems of reductionism, the use of models, and the role of clinical insight.

In general the authors apply the insights of the modern philosophy of science to the language of psychology. The book achieves this pedagogic aim. Its restatement of the philosophy of science as applied to psychology is interesting, lucid, and well balanced. The authors talk about how to talk about psychological words and theories. Despite the value of this book in summarizing current opinion regarding the applications of the philosophy of science to psychology, the authors have neglected the more difficult task of applying these concepts to actual psychological theories. The crucial question lies in the analysis of real theories such as psychoanalytic and learning theory, and the discussion of such points as the following: Does the language of Freudian psychology refer to empirical events with sufficient response invariance, does it allow for the deduction of testible hypothesis, and are its theoretical constructs logically consistent; Does Lewinism theory explain any empirical event parsimoniously or is it merely a belabored and ambiguous description of the event allowing in principle no prediction? In failing to come to grips with any actual psychological language the book is nonspecific and the usefulness of this approach to the analysis of psychological systems (being unattempted) remains unproven.

JOHN M. DAVIS

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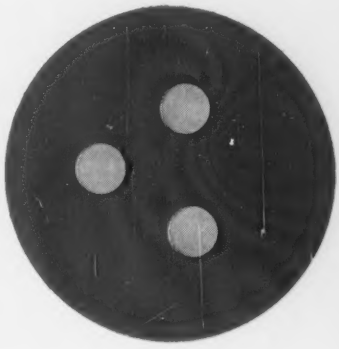

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